

MONARCH BUTTERFLY MATING ECOLOGY AT A MEXICAN  
OVERWINTERING SITE: PROXIMATE CAUSES OF NON-RANDOM  
MATING

BY

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by

Tonya Van Hook

Dedicated, with love, to my parents and my husband

...and to the insects that are largely responsible  
for the beauty and bounty we know as life

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## TABLE OF CONTENTS

ACKNOWLEDGMENTS.....	iv
ABSTRACT .....	xii

### CHAPTERS

1 INTRODUCTION .....	1
Introduction to Mating Ecology.....	1
Mating Ecology of Butterflies.....	3
Mating Ecology of Monarch Butterflies.....	6
This Study.....	8
General Prediction.....	8
Small Male Mating Advantage.....	9
Mating Biology of Females at the Overwintering Site.....	9
Rejection of The Hypotheses that Female Nutrient Need has Been a Major Selective Force Accounting for the Extreme Overwintering Densities.....	10
Conclusions.....	11
2 NON-RANDOM MATING IN MONARCH BUTTERFLIES OVERWINTERING IN MEXICO.....	13
Introduction.....	13
Methods.....	16
General Population: Non-mating Butterflies, 1985.....	17
Mated Butterflies, 1985.....	18
Tests for Bias of Mated Pairs, 1986.....	19
Comparisons of Butterflies Collected at Various Points During Ground-phase Precopulatory Interactions, 1986.....	20
Statistical Analyses.....	22
Results.....	22
General Population, 1985.....	22

Comparisons of In Copula Mated Pairs versus Non-mated Butterflies of the General Population, 1985.....	24
Tests for Bias of Mated Pairs, 1986.....	25
Comparisons of Butterflies Collected at Various Points During Ground-phase Precopulatory Interactions, 1986.....	26
Discussion.....	28
Female Perspectives.....	31
Postulated Alternate Male Reproductive Behaviors.....	34
Male Mate Discrimination at Mexican Overwintering Sites.....	36
Conclusions.....	37
 3 PROXIMATE CAUSATION OF SMALL, WORN MALE MATING ADVANTAGE IN OVERWINTERING MONARCHS .....	47
Introduction.....	47
Hypothesis 1: Large, Good Condition Males Do Not Attempt to Mate.....	51
Hypothesis 2: Small Male Takedown Advantage in Aerial Pursuit.....	52
Methods.....	53
Hypothesis 1: Large, Good Condition Males Do Not Attempt to Mate .....	53
1985 Male activity versus phenotype comparisons.....	53
1988 Male reproductive tract development versus mating status.....	53
Reproductive tract dissections.....	55
Lipid analysis.....	56
Statistical analyses.....	57
Hypothesis 2: Small Male Takedown Advantage in Aerial Pursuit.....	57
Rearing.....	57
Tests of possible negative effects of larval starvation on adult vigor .....	58
Experimental design.....	61
Large flight cage trials.....	63
Small cage trials.....	64
Statistical analyses.....	65

Results.....	65
Hypothesis 1: Large, Good Condition Males Do Not Attempt to Mate.....	65
1985 Male activity versus phenotype comparisons.....	65
1988 Male reproductive tract development versus mating status.....	66
Hypothesis 2: Small Male Takedown Advantage in Aerial Pursuit.....	70
Discussion.....	72
Hypothesis 1: Large, Good Condition Males Do Not Attempt to Mate.....	72
1985 Male activity versus phenotype comparisons.....	72
1988 Male reproductive tract development versus mating status.....	72
Hypothesis 2: Small Male Takedown Advantage in Aerial Pursuit.....	76
General Discussion.....	80
The ontogeny of male mating tactics.....	81
Do non-diapausing monarchs remigrate?.....	88
Conclusions.....	91
 4 THE USE OF BURSA COPULATRIX DISSECTION AND ABDOMINAL PALPATION TO ASSESS FEMALE MONARCH BUTTERFLY MATING STATUS.....	104
Introduction.....	104
Methods.....	106
Bursa Copulatrix Dissections.....	106
Female Abdominal Palpations.....	108
Results.....	104
Designation of Old Matings Versus Virgins Using Bursa Copulatrix Dissections.....	109
Palpation Accuracy and Spermatophore Degradation through Time.....	112
Palpation versus Bursa Copulatrix Dissection.....	114
Discussion.....	115
Bursa Copulatrix Dissections.....	115
Abdominal Palpations.....	116
Spermatophore Degradation as a Means to Differentiate Between Autumn and Overwintering Female Mating.....	118
Conclusions.....	119

5	TIMING AND FREQUENCY OF FEMALE MONARCH MATING AS RELATED TO MEXICO OVERWINTERING ECOLOGY.....	126
	Introduction.....	127
	Monarch General Overwintering Biology.....	127
	This Study.....	129
	Methods.....	130
	General Description of the Site.....	130
	Wing Condition Rankings.....	131
	Bursa copulatrix dissections.....	132
	Female abdominal palpations.....	133
	Results.....	134
	Overwintering Colony Characteristics.....	134
	Yearly descriptions of colony movement and sub-colony formation.....	136
	Fire and colony dynamics.....	139
	Mating Activity.....	140
	Frequency of Female Mating.....	142
	Changes in female mating status through time: Bursa copulatrix dissection: 1985.....	145
	Changes in female mating status through time: Abdominal palpation: 1985-1988.....	146
	Female Activity Versus Mating Status.....	148
	Female Condition Versus Mating Status.....	149
	Discussion.....	150
	Overwintering Colony Characteristics.....	150
	Mating Activity.....	153
	Female Mating Before the Mass Mating Event.....	154
	Frequency of Female Mating During the Mass Mating Event.....	156
	Polyandry.....	158
	Female Activity and Condition versus Mating Status.....	161
	Summary of Bursa Copulatrix Dissection and Palpation Results.....	166
	Frequency and Degree of Multiple Mating in Different Life Cycle Stages and in Different.....	166
	Possible Proximate Explanations for Differences in Frequency and Degree of Mating in Mexico Versus California Overwintering Females.....	168
	Environmentally-Induced Variation in the Timing of Female Mating During Their Migratory Life History Cycle.....	171

Environmentally-Dependent Costs and Benefits of Different Monarch Mating Tactics.....	173
Summary of Comparisons of Mexico, California and Australia Overwintering Mating Ecology.....	174
6 POLYANDRY AS A SELECTIVE AGENT IN THE EVOLUTION OF MONARCH OVERWINTERING AGGREGATIONS.....	187
Introduction.....	187
Method.....	191
Results.....	192
Discussion.....	194
Multiple Mating as a Primary Selective Pressure for Monarch Overwintering Aggregations.....	194
Frequency and Degree of Mexico Overwintering Female Mating.....	196
Male Investment in Spermatophores.....	198
Potential for Spermatophores to Account for Lipid Weight Increases Observed in Late Overwintering Females.....	199
Potential for Spermatophores to Contribute Significant Cardenolide Quantities to Late Overwintering Females.....	203
Other Evidence Against Polyandry as a Selective Agent in the Evolution of Monarch Overwintering Aggregations.....	204
Conclusions.....	208
7 DISCUSSION.....	211
REFERENCES.....	223
BIOGRAPHICAL SKETCH.....	242

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By

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December 1996

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Monarch butterflies are useful organisms for studying insect mating system ecology due to large male investments in spermatophores and their unusual life history strategy. Unlike in summer breeders, the overwintering mating event 1) involves a single generation, 2) is removed from larval resources in space and time, 3) follows reproductive diapause, and 4) is explosive and occurs under extremely high population densities.

Following current mating ecology theory, I predicted that overwintering monarchs should mate assortatively, i.e. large, good condition individuals should mate preferentially; leaving small, poor



condition individuals to pair. This was based on evidence that 1) the reproductive rate of both sexes are expected to be constrained by material investments and the brief mating period, 2) butterfly phenotype is expected to indicate their worth as mates and their ability to exercise mate choice, and 3) the dense overwintering populations enhance opportunity for mate choice.

As predicted, *in copula* females were larger, heavier and in better condition than females in the general population. Male differential takedown apparently accounts for the large, good condition female mating advantage. Unexpectedly, small, worn males were over represented in mating pairs. Reproductive tract dissections showed that mating males were primarily old individuals that did not overwinter in reproductive diapause and had little future mating opportunity. Female choice was not supported and may be constrained physiologically by their relatively longer state of diapause and/or behaviorally by the male takedown strategy.

Dissections of the bursa copulatrixes showed that females mate before, during and after overwintering, but the frequency of mating and the degree of multiple mating was low at the Mexico site. The costs and benefits of mating at the overwintering sites must therefore be viewed in terms of lifetime reproductive success.

In conclusion, my research demonstrates non-random mating at a Mexico overwinter site and emphasizes the importance of environmental conditions in determining both phenotypic-dependent mating behaviors and their reproductive consequences. I encourage comparative studies of monarch mating patterns during different

stages of the life history cycle and in different populations to determine the evolutionary and demographic effects of these mating patterns.

## CHAPTER 1 INTRODUCTION

### Introduction to Mating Ecology

Darwin (1871) defined sexual selection as evolutionary processes that account for traits associated with mating success. He noted sex differences characterized by competition among males for mates and choosiness in mate selection by females. Darwin recognized that both ecological conditions and phylogenetic history are important determinants of sex differences in reproductive behavior. He considered sex-role reversal and male mate choice as special cases explainable in terms of sexual selection theory.

Fisher (1958) initially formalized sexual selection theory, and more currently numerous authors (e.g., references in Bateson 1983, Smith 1984, Bradbury and Anderson 1987, Clutton-Brock 1988) refined Darwin's early ideas into testable hypotheses. Trivers (1972) made explicit that sex differences in intensity of sexual selection result more generally from differences between the sexes in parental investment.

Following Trivers, the reproductive rate of females is generally limited both by pre- and postzygotic investments of substantial time, energy and resources in their offspring. Females therefore best enhance their reproductive fitness by mating with males that offer superior resources and/or genes. Because male pre- and postzygotic

investments are usually minimal, male reproductive rate is limited indirectly by the number of females they can successfully inseminate. Choosy females therefore can act as a limiting resource in male reproductive success resulting in male-male competition for mates (Bateman 1948, Williams 1966, Trivers 1972).

As male investments or ecological constraints to polygyny increase, males may become limited in the number of females they can inseminate (Knowlton 1979, Nakatsuru and Kramer 1982, Clutton-Brock and Parker 1992). Such males are predicted to be selective in mate choice (Trivers 1972, Dewsbury 1982, Gwynne 1984 a, b). Thus, even in the absence of sex-role reversal, males that invest heavily may both compete for mates and show mate choice (Halliday 1983).

Male reproductive success is not equivalent to mating success *per se*, but rather depends on the number of eggs he successfully fertilizes. Female insects store sperm that are generally long-lived (Davey 1965, Parker 1970), and sperm derived from different males likely vary in their reproductive value to females. Hence, there is potential both for competition among sperm from different ejaculates and female control of which sperm fertilize her eggs (Parker 1970, 1984, Eberhard 1996). The mechanisms and arena of sexual selection are therefore extended in species in which females multiply mate and store sperm (e.g. references in Smith 1984 and Eberhard 1996). The diversity of female insect mating patterns, sperm storage structures and sperm utilization strategies increase their value as research subjects to understand adaptive mating behaviors in general.

Emlen and Oring (1977) emphasized the need to view adaptive mating behaviors within the context of the ecological constraints and opportunities under which sexual selection acts. Their basic argument was that the ability of males to control access to mates is an important determinant of the intensity of sexual selection, and that this ability reflects environmental conditions such as the spatial and temporal distribution of females or the resources they require. They also pointed out that, if we are to understand the evolution of mating systems, we must consider the impacts of different mating systems on population genetics and demographics.

Finally, selective pressures differ in time and space, can depend on the individual's phenotype, and may be frequency dependent. We therefore expect adaptive alternative mating behaviors (Alcock 1979, Cade 1980, Rubenstein 1980, Waltz 1982, Dunbar 1982, Gross 1982, Austad 1984, Dominey 1984, Waltz and Wolf 1984, Caro and Bateson 1986, Gardner et al. 1987). Until recently individual variation in behavior has generally been dismissed as statistical error or aberrations of the species-typical pattern (Dunbar 1982). The development of game theory (Maynard Smith 1982) and the concept of evolutionarily stable strategies (Parker 1982) have been important in detecting and understanding frequency dependent mating behaviors.

### Mating Ecology of Butterflies

Butterflies were prominent in Darwin's development of sexual selection theory, primarily because of the importance of visual cues

used in mate recognition and intrasexual interactions (see Chapter 11, in Darwin 1871). Butterflies have recently become valued as study organisms due to their large investments in reproduction by males. These investments are expected to limit male reproduction rate and could thereby lead to male discrimination in their choice of mates. Because females utilize materials provided in spermatophores to enhance their reproductive success and males vary in their ability to provide these materials, females also are expected to discriminate in their choice of males (Rutowski 1984).

Female lepidopterans store sperm, and they may also utilize spermatophore-derived nutrients for somatic maintenance and egg production. In polyandrous species females may therefore re-mate multiple times to gain nutrients and perhaps to selectively fertilize their eggs. Female remating is disadvantageous to males since the last male to mate generally fathers most of the offspring. There is therefore a potential conflict of interest (*sensu* Parker 1979) between the sexes in female sperm and nutrient usage. The outcome of this conflict of interest is thought to account for spermatophore size and content, and the extent of female multiple mating (Drummond 1984, Parker and Simmons 1989, Boggs 1995). The relative importance of direct reproductive benefits through paternal investments in offspring, female choice of males for genetic or material benefits, and male investments to avoid sperm competition by preventing or delaying female remating remain controversial (Alexander and Borgia 1979, Gwynne 1984a, 1986, Knowlton and Greenwell 1984, Wickler 1985, 1986, Parker and Simmons 1989, Boggs 1995, Eberhard 1996).

The outcome of conflicts of interest between the sexes also depends on the relative reproductive costs and benefits between the sexes and on which sex has relatively more control over the act of mating and the outcome of sperm competition (Ridley 1990, Eberhard 1996). Understanding the adaptive significance of differences in butterfly mating behaviors is further complicated by the fact that spermatophore function in sperm competition, paternal investment and mate acquisition are not mutually exclusive.

Butterfly mating ecology is thought to both shape and be shaped by the impact of male investments on male and female reproductive success (Rutowski 1984, Forsberg 1987, Boggs 1995). The ecological conditions under which monarchs mate during the different parts of their life history cycle among different overwintering populations. Monarchs also show considerable variation in spermatophore size; both among individuals and through a male's lifetime (Oberhauser 1988, 1989, Svård and Wiklund 1988a). They therefore offer a unique opportunity to study the interplay of male investments and ecological conditions in the evolution of an insect mating system. Monarch mating patterns, in turn, influence population demographics and may be crucial in maintaining their migratory life history strategy. Understanding the proximate causes of these patterns is therefore prerequisite to developing effective conservation plans for preserving their spectacular migration, which is considered an endangered phenomenon (Brower and Malcolm 1991).

### Mating Ecology of Monarch Butterflies

Monarch butterflies east of the Rocky mountains migrate to the central highlands of Mexico where they overwinter in a few isolated sites (Calvert and Brower 1986). They characteristically spend the winter in a state of reproductive diapause lasting approximately 5 months. Before remigration, and in association with males breaking reproductive diapause, they mate in an exponentially increasing mass mating event. Mating occurs within the extremely dense overwintering aggregations, estimated at approximately 10 million butterflies per ha.

Stored energy reserves are depleted during the overwintering period and a long distance remigration precedes oviposition at sites in the southern U.S. (Brower 1995). Female nutrient need is therefore expected to be high and crucial to her reproductive success. Females use male derived nutrients for somatic maintenance and egg production (Boggs and Gilbert 1979, Wells et al. 1993). Higher quantity or quality of male-derived materials has been shown to increase female reproductive success (Oberhauser 1989, in press, but see Svård and Wiklund 1988 a). Furthermore, size, age and mating history effect spermatophore size (Oberhauser 1988). Females are therefore expected to prefer large, young, virgin males since these males are expected to provide superior material benefits.

Male rate of mating is expected to be limited by their large investments in spermatophores (Oberhauser 1988), their prolonged copulation times (Svård and Wiklund 1988b), and the brevity of the spring mass mating event (Hill et al. 1976). Such constraints may



increase the reproductive benefits of male choosiness among potential mates, especially under high population densities (Clutton-Brock and Parker 1992). Female size is positively related, and female age is negatively related, to her fecundity (Zalucki 1981, Oberhauser in press). Large, young females are also expected to be better migrators than small, old females (Gibo and Pallett 1979). Males that mate preferentially based on female large size, young age are expected to gain in reproductive success. Even with last male sperm precedence in fertilization, males are also expected to benefit from preferring virgins when mate location costs are low.

The monarch butterfly has a simple aerial capture rather than a complex courtship as described in the closely related queen butterfly (*Danaus gilippus benenice* (Cramer), Brower et al. 1965). Furthermore, hairpencils used to waft pheromones, are much smaller in the monarch compared to the queen, and their use is not prerequisite to mating success (Pliske 1975). The consistent struggle by females in precopulatory interactions led Rothschild (1978) to describe the mating pattern as rape, an unfortunate term. The struggle between a male and female can be lengthy and many attempted copulations are unsuccessful.

Monarch courtship behavior in a summer breeding population has been described in detail by Pliske (1975). Hill et al. (1976) described mating at a California overwintering site and found no notable differences in behavior from those reported by Pliske. The male takedown strategy may provide a mechanism to exercise mate choice. Female evasion and resistance may also select for males that are likely to provide superior material benefits.

## This Study

### General Prediction

Five factors are important determinants of mate choice to enhance material benefits: 1) the reproductive rate of the choosy sex is limited, 2) mate choice increases reproductive success, 3) the choosy sex has the capacity to exercise choice, 4) potential mates vary in their ability to provide benefits and phenotypic cues reliably indicate this variation, and 5) the environment is conducive to mate choice. Based on the natural history outlined above, these factors are expected to favor mate choice in both sexes of overwintering monarch butterflies. I therefore predicted that monarchs should mate assortatively, i.e. large, good condition individuals should mate preferentially; leaving small, poor condition individuals to pair among themselves. This assortative mating pattern arises by 2 mechanisms. First, both sexes are expected to choose their mates based on cues indicative of material benefits, and large size and good condition are correlated with ability to provide these benefits. Second, large, good condition individuals of both sexes are expected to be best able to exercise mate choice. Male scramble competition may occur simultaneously with male mate choice in males with constrained reproductive rates. Such competition is expected to enhance positive assortative mating.

### Small Male Mating Advantage

I found that *in copula* females were larger, heavier and in better condition than females in the general population. However, mating males were characteristically small and worn. They were not of the phenotype expected to be selected either by male-male competition or by female choice. I found no evidence for female mate discrimination, and no evidence for small male takedown ability that could account for the small males' mating success. Reproductive tract dissections of both mated males and those collected from overwintering clusters suggested that small, old, non-diapausing individuals made up most of the male mating population. Post-diapause reproductive tract development in males collected from clusters was associated with the increase in the intensity of the mating event, and was also found in a sub-set of mating males. Behavioral changes associated with post-diapause reproductive tract development may therefore account for some of the overwintering male matings.

### Mating Biology of Females at the Overwintering Site

Female monarchs store sperm, utilize spermatophore-derived nutrients, mate multiply and the sperm of the last male to mate has precedence in paternity of the offspring. It is therefore important to know when in the life cycle female monarchs receive spermatophores in order to understand 1) male and female mating strategies, 2) remigration population demographics, and 3) selective

pressures that shape and maintain the migration-diapause life history strategy.

I therefore determined the proportion of females that mate, and the degree of polyandry, both before and during the mass mating period. My primary findings were that 1) females mate during the southward migration and/or early on in the overwintering period, 2) mass mating activity is restricted to the last few weeks of overwintering, increases exponentially, and most matings may occur just before the main exodus from the overwintering site, 3) female utilization of spermatophores may increase as the mass mating activity begins, 4) most females leave the overwintering site without mating during the mass mating event, but not necessarily as virgins, and 5) multiple mating during the mass mating is rare, but females that had previously mated may mate again during the mass mating period. These findings are compared to those of other workers studying California and Australia overwintering monarchs, and summer breeders. I also dissected remigrant females that were collected in the southern U.S. in the early spring and found that remating by overwintering females is apparently universal and multiple.

Rejection of the Hypothesis that Female Nutrient Need has Been a  
Major Selective Force Accounting for the Extreme  
Overwintering Densities

I tested predictions based on the hypothesis put forth by Wells et al. (1990) and Wells et al. (1993), that female nutrient need has

been a major selective force accounting for the extreme density of the overwintering colonies. Although female nutrient need may be an important determinant of female mating patterns, I found no evidence that increased opportunity for polyandry has been an important selection agent accounting for the extreme densities of Mexico overwintering aggregations.

### Conclusions

I review my results in light of those of other workers studying monarch mating biology. Based on this review, I suggest that environmental conditions experienced among individuals, locations, and phase of the life cycle impose variation on monarch migration and reproductive physiology. For example, late summer overlapping generations distributed over the continental U.S. are expected to experience a variety of daylengths, temperatures, food plant quality, and larval competition. Variation in these conditions lead to variation in reproductive state through neuroendocrine responses, and thereby define the late summer and/or migratory reproductive effort. Individuals may be more or less flexible in their response to changes in environmental conditions, but successive physiological and behavioral responses are likely to limit future behavioral options. The collective population variation is, in turn, a primary determinant of the frequency, degree, and evolutionary and demographic significance of overwintering mating.

I suggest that differences in environmental factors alone can account for differences in mating patterns observed among different

overwintering populations, and during different stages of the monarch's life history cycle. The evolutionary and demographic effects of these mating patterns await further study. My research suggests that mating patterns, female sperm and nutrient usage, and environmental and phenotypic constraints must be considered before, during and after the overwintering mass mating event.

## CHAPTER 2

### NON-RANDOM MATING IN MONARCH BUTTERFLIES OVERWINTERING IN MEXICO

#### Introduction

Monarch butterflies, *Danaus plexippus* (L.), from eastern North America migrate each autumn to a few tightly packed aggregation sites in the volcanic highlands of central Mexico (Calvert and Brower 1986, Calvert and Lawton 1993). A brief mating period occurs just before colony breakup and remigration from these sites in early spring. Migration and aggregation behaviors bring nearly every adult in the entire population into close contact during the time of mating. Such a concentration of adults, at up to 10 million per ha (Brower et al. 1977), presents a unique opportunity for mate discrimination before colony breakup and the onset of remigration.

Male monarchs are predicted to be selective in their choice of females for 4 reasons: 1) they invest up to 10% of their body weight in each spermatophore transferred (Oberhauser 1988, Svård and Wiklund 1988); 2) the opportunity for polygyny may be limited by a brief period, long copulation times, and possible long male intermating intervals due to accessory gland depletion (Knowlton 1979, Oberhauser 1989); 3) egg production, egg laying lifespan, and migration ability are related to female size and condition (Brower and Glazier 1975, Suzuki 1978, Gibo and Pallett 1979, Zalucki 1981,

Oberhauser in press); and 4) the mating behavior involves male capture and takedown of females in an aerial pursuit (Hill et al. 1976, Pliske 1975, Urquhart 1987).

Female monarchs are predicted to be selective in their choice of males for the following reasons: 1) females incorporate nutrients provided by males through spermatophore transfer for both egg production and somatic maintenance (Boggs and Gilbert 1979, Wells et al., 1993); 2) female nutrient needs are expected to be especially high in late overwintering females that must remigrate before ovipositing; and 3) males vary in their ability to provide nutritive benefits, as measured by spermatophore weight (Oberhauser 1988).

Polyandry is an unlikely mechanism to significantly boost Mexican overwintering female nutrient resources, as has been proposed (Herman 1981, Brower 1985, Wells et al. 1990, Wells et al. 1993). I found that multiple mating was rare in a Mexico overwintering female population. Furthermore, unlike summer breeders and California overwintering females (Hill et al. 1976, Oberhauser 1989, Oberhauser 1992, Zalucki 1993), the degree of polyandry was extremely low in those females that mated more than once (Chapter 5). Polyandry may be constrained in Mexico overwintering females by unique environmental conditions, male strategies, or female reproductive physiology. Alternatively, females may adaptively limit multiple mating in Mexico relative to other sites. For example, the weight of multiple spermatophores may cause wingloading remigration costs that are not incurred when host plants are nearby. Regardless, nutrient limited females that rarely mate multiple times should prefer to mate with males that provide



larger or higher quality spermatophores (Rutowski et al. 1987, but see Oberhauser 1989).

Spermatophore size, and therefore amount of material benefits available to females, is positively correlated with male size and negatively correlated with male age and mating history (Oberhauser 1988). Phenotypic cues indicative of the male's capacity to provide material benefits, including size, weight and condition, should be used as cues for female mate choice (Rutowski 1984, Rutowski 1985). Reproductive advantages of mating with males that produce larger spermatophores have been demonstrated in *Colias eurytheme*. Rutowski et al. (1987) showed that larger male contributions led to increased female longevity, egg-laying rate, and lifetime egg production (but for evidence against this possibility in monarchs see Svård and Wiklund 1988, and Oberhauser 1989).

Finally, assuming that size has a significant heritability (Arango-V. 1996), both males and females may potentially gain from mating with large partners. This is especially relevant in a long-distance migratory insect such as the monarch. Monarchs show considerable variation in wing size and wing condition. Examples of the extremes of wing size are shown in Figure 2-1, and examples of good, medium and poor wing condition are shown in Figure 2-2.

Brower et al. (1977) (also see Eanes and Koehn 1978) described the mass mating activity at Mexican overwintering sites as panmictic with respect to geographic origin, but did not address non-random mating based on phenotype. In this paper, I test the hypothesis that extremely high population densities and the brief mating period at Mexican overwintering sites, together with male investments and

material benefits available to both sexes, could lead to positive assortative mating via both male and female choice (i.e., large, good condition males would mate preferentially with large, good condition females and *vice versa*). My approach was to compare non-mating and mating butterflies with respect to body weight, wing length, and wing condition during the mass mating period at a Mexican overwintering site in mid-February through late March 1985.

In 1985 I collected mated pairs within the colony boundaries and within reach of my butterfly net, which could have biased the sample toward males that could not readily fly higher or farther with females in tandem flight. To test for this possible sampling bias, I tested male ability to carry females in tandem flight. Secondly, I compared male and female phenotypes among 4 sequential precopulatory phases to determine when in the sequence non-random pairing might originate. These tests were conducted at the same overwintering site during the mass mating period in 1986. Taken together, the 1985 and 1986 data provide strong evidence for non-random mating, but in a direction that was not anticipated.

### Methods

The site of the overwintering colony was similar in 1985 and 1986 and was located at approximately 3,000 m elevation on the Sierra Chincua, Michoacan, Mexico, at 19°41'N, 100°18'W (Calvert and Brower, 1986). In both years the colony covered approximately 1 ha and was nestled between two small ravines. In 1985, data were collected from 11 February through 25 March, concurrent with the

timing of mating activity. In 1986, data were collected from 1 -24 March, after mating began in mid-February. In both years nearly all the butterflies had dispersed from the overwintering area by the end of the data collection period.

#### General Population: Non-mating Butterflies, 1985

Three phenotypic characters (wet weight, wing length, and wing condition) were measured in four categories of non-mating monarchs (i.e., butterflies in or near the colony that were not mating when collected). The four categories were 1) actively flying within 3 m of the ground, 2) at water or mud sources (this category is hereafter referred to as drinking, though proboscis contact was not determined), 3) clustered at up to 6 m above the ground (hanging immobile on tree trunks or leaves), and 4) nectaring at flowers.

Butterflies were netted and immediately placed in glassine envelopes. Within 6 h they were stored in plastic bags on ice until being measured the following day. Wet weight was measured to the nearest milligram, and forewing length was measured to the nearest 0.5 mm along the costal margin from base to apex. Wing condition was rated subjectively as good, medium, or poor (1, 2, and 3, respectively) based on wing scale loss, fading, scratches, and degree of tattering along the margin. To ascertain that there were no significant differences for either males or females among the 4 categories of non-mating monarchs, I performed separate Kruskal-Wallis tests for each phenotypic character. To control for changes

with time, the data were divided into two subgroups: early (collected before 5 March) and late (collected on or after 5 March).

### Mated Butterflies, 1985

*In copula* mating pairs were collected from 11 February to 25 March, usually in the afternoon when they were most abundant. Because they were not found outside the colony and were generally restricted to scrubby vegetation, collections were made only inside the cluster area and within 3 m of the ground. Each mated pair was stored together overnight in a glassine envelope on ice. I allowed the pair to remain *in copula* until they separated, almost invariably the following morning. They were then individually weighed, measured, and rated for wing condition. Mated females were palpated to determine presence or absence of a freshly transferred spermatophore by gently applying pressure between the thumb and index finger while feeling along the abdomen from anterior to posterior for a swollen bursa copulatrix. Phenotypic characters of mated males and females were compared with those from the general overwintering population. Using female abdominal palpations as the criterion for distinguishing mating status, males and females that successfully transferred spermatophores were compared with those that were unsuccessful.

Tests for Bias of Mated pairs, 1986

In 1985, mated pairs were collected exclusively within the colony boundaries and within reach of my butterfly net. Because the normal behavior of mating pairs is to fly off in tandem after coupling and then settle on vegetation, it was possible that the mated pair sample of 1985 involved only those pairs that did not or could not fly out of the colony or those that crawled up nearby vegetation and became quiescent without a tandem flight. To test this possibility, I made two indirect control samples in 1986.

First, I compared quiescent mated males collected in the same manner as in 1985 to males that successfully coupled during ground-phase interactions, i.e., those that had just attained genital coupling and which I caught before post-nuptial flight. This latter sample would not be biased toward weak fliers. Second, I tested whether just-coupled males could take flight and carry the female in tandem flight. Upon capture, each pair was immediately placed into a glassine envelope and transported to an open area near the colony, approximately 50 m in diameter with grass and sparse scrubby vegetation and surrounded by dense stands of trees. Pairs that uncoupled during transport were not tested. I tested 13 pairs on 5 and 16 March between 1415 and 1615 h. Both days were clear and sunny with mild to strong breezes and ambient temperatures were at least 16°C (i.e., above flight threshold; Masters et al., 1988; Alonso-M. et al. 1993). Each male-female pair was carefully measured and ranked for wing condition before release without uncoupling them. Males that were able to carry females out of the open area within 2

min. were scored as successful, regardless of whether flight was directly from the ground or followed climbing up nearby vegetation. Unsuccessful males either failed to take flight directly or climbed up vegetation and then became quiescent for at least 2 min. Males in the unsuccessful group were compared with those that successfully flew away, with respect to wing size and wing condition.

Comparisons of butterflies Collected at Various Points During  
Ground-phase Precopulatory Interactions, 1986

For purposes of this study, the mate pairing sequence can be summarized as follows: Males pursue females in aerial flight. If successful at capturing the female, the male folds his wings and together the pair falls to the ground. On the ground, males attempt to couple with the assistance of genital claspers. Females appear to struggle throughout the ground-phase precopulatory interaction but they rarely escape. A male may leave a female at any point during the ground-phase interaction. If successful genital coupling occurs, the female becomes quiescent and the male carries her away in a postnuptial flight. Males also sometimes pursue and takedown other males. Ground-phase male-male interactions appear indistinguishable from male-female interactions, except the pursuing male always eventually abandons the male it has taken down.

To determine when in the mating sequence phenotypic non-random pairing might originate, monarchs from the 4 ground-phase precopulatory categories were collected. These samples were handled in the same fashion as mated pairs. Collections were made from 1-25 March between 1100 and 1600 hrs and included 1)

attempted copulations, pairs in which a male held a female while probing her genital area with his abdomen (these were collected upon sighting without allowing the precopulatory interaction to continue and, therefore, do not distinguish successful from unsuccessful mating attempts); 2) just-coupled butterfly pairs, as described above; 3) abandoned individuals, either male or female butterflies left by their potential mates during ground-phase interactions; and 4) homocourtships, male-male pairs in which one male was attempting copulation with a second male. The homocourtship category is identical to the attempted copulation category except the pursued individual was a male.

Males and females from the attempted copulation group were compared to the general population to determine whether all male phenotypes successfully takedown females and whether they do so randomly. Attempted copulation pairs were also compared to just-coupled pairs to determine if all pairs that fall in aerial takedown are equally likely to couple successfully. To assess whether females abandoned during precopulatory interactions were distinct from those that successfully coupled, females left by their potential partners during ground-phase courtship were compared with those that had just coupled successfully. Finally, sexually aggressive males and males with which they were attempting to copulate were compared to each other, to the general population, and to *in copula* mated males to determine if these males were of distinct phenotypes. Females in all categories were palpated to determine recent mating status, using the method described above for mated females.

### Statistical Analyses

For data analyses I used SAS (1985). Data were tested for normality with the Kolmogorov D test. Because the data in several cases were not normally distributed, I used nonparametric statistics: Wilcoxon two-sample rank-sum (two-tailed tests), Kruskal-Wallis multiple-sample (chi-square approximation), and Spearman's rank-order correlation coefficient.

### Results

#### General Population, 1985

Mating was first observed on 11 February, gradually increased in frequency through 25 February, and remained high through the end of the overwintering period, except during unusually cool and overcast days. The general overwintering mating natural history observed in 1985-1988 is reported in Chapter 5. Almost all the butterflies had dispersed from the overwintering colony area by 19 March.

The four general population sampling categories of actively flying, drinking, clustered, and nectaring varied widely in sample sizes and sex ratios across collection dates (Table 2-1). In early season collections, both sexes differed in wet weight among sampling categories, but neither sex differed in forewing length or wing condition (Table 2-2). In late season samples, females differed



among sampling categories in wet weight and wing condition, while males showed no differences in any of the 3 characters measured. Phenotypic differences among sampling categories were not consistently different for either males or females in early- and late-season samples. For further statistical comparisons, I chose to combine all four sampling categories listed in Table 2-1 to represent the general non-mating overwintering population.

In the general population, male and female mean wet weight and mean wing size differed among good, medium, and poor wing condition groups. Larger mean wet weights and wing sizes were associated with better wing conditions (Table 2-3). These trends were consistent in early and late samples (only male forewing length was not significantly positively associated with wing condition in the early-season sample;  $X^2 = 3.95$ ,  $p = 0.14$ ,  $df = 2$ ,  $n = 233$ ). Mated individuals showed the same trend with heavier wet weights and longer wing lengths associated with better wing conditions (Table 2-3). Early-and late-season samples showed the same trend except that in the early season, female wet weights and wing sizes did not differ among the three wing condition categories ( $\chi^2 = 3.39$ ,  $p = 0.18$ ,  $df = 2$ ,  $n = 122$ ;  $X^2 = 1.83$ ,  $p = 0.40$ ,  $df = 2$ ,  $n = 125$ , respectively).

As determined by abdominal palpation, 90% of the 450 *in copula* mated pairs collected in 1985 successfully transferred spermatophores. Males that were unsuccessful in transferring spermatophores weighed less and were in worse condition than successful males (weight:  $Z = -2.22$ ,  $p = 0.03$ ,  $n = 434$ ; condition:  $Z = -3.53$ ,  $p = 0.0004$ ,  $n = 447$ ), while they did not differ in wing length ( $Z = -1.05$ ,  $p = 0.30$ ,  $n = 444$ ). The test for weight difference is probably

conservative because males that successfully transferred spermatophores had just lost the weight of a fresh spermatophore, yet they still weighed significantly more than unsuccessful males. In contrast, successful spermatophore transfer did not reflect female wet weight ( $Z = -0.81$ ,  $p = 0.42$ ,  $n = 442$ ), wing length ( $Z = 1.56$ ,  $p = 0.12$ ,  $n = 450$ ), or wing condition ( $Z = -0.81$ ,  $p = 0.42$ ,  $n = 450$ ).

#### Comparisons of *In Copula* Mated Pairs versus Non-mated Butterflies of the General Population, 1985

Mated males weighed less, had shorter wings, and were in worse condition than non-mating males in the general population (Table 2-4). Mated females showed opposite trends relative to non-mating females. They were heavier, larger, and in better condition. These differences between mating and non-mating individuals were of such magnitude that mated monarchs showed opposite male-female weight, size, and wing condition trends relative to non-mating monarchs. Males in the general population weighed more and had longer wings than females, while they did not differ in wing condition. In contrast, mating males weighed less, were smaller, and were in poorer condition than mating females (Table 2-4).

Within the mating population, there was no evidence of negative assortative pairing by wet weight or wing length. Spearman rank-order analyses showed no significant correlation in wet weight ( $r_s = 0.06$ ,  $p = 0.23$ ) or wing length ( $r_s = -0.01$ ,  $p = 0.78$ ) between mated pairs. Male and female wing condition showed a slight but significant negative correlation ( $r_s = -0.17$ ,  $p = 0.0004$ ).

Phenotypic characteristics of mated monarchs changed through the mating period. Males mating early weighed less and were in worse condition than those mating after 5 March ( $Z = 3.11$ ,  $p = 0.002$ ;  $Z = 4.42$ ,  $p = 0.0001$ , respectively). However, male wing size did not differ between early and late mating samples ( $Z = -1.39$ ,  $p = 0.17$ ). Mated females showed different trends. Those that mated early weighed more and had longer wings than those mating late ( $Z = 3.50$ ,  $p = 0.0005$ ;  $Z = 3.07$ ,  $p = 0.002$ , respectively). Unlike mated males, there were no differences in female wing condition with time ( $Z = -0.17$ ,  $p = 0.86$ ).

In contrast, the general population changed little with time. Non-mating males collected early versus late in the breeding period did not differ in wet weight ( $Z = 0.57$ ,  $p = 0.57$ ), wing size ( $Z = 1.40$ ,  $p = 0.16$ ), or wing condition ( $Z = 0.42$ ,  $p = 0.68$ ). Similarly, wet weight and wing size of early and late collections of non-mating females did not differ ( $Z = -0.85$ ,  $p = 0.40$ ;  $Z = 1.24$ ,  $p = 0.22$ , respectively). Only female wing condition changed significantly through the breeding period ( $Z = 3.19$ ,  $p = 0.001$ ). Mating monarchs more closely resembled the general non-mating overwintering population as the breeding season progressed (Table 2-5).

#### Tests for Bias of Mated Pairs: 1986 Sample

Wet weights, wing sizes, and wing conditions of *in copula* males and females did not differ from those that had just coupled. This suggests that the 1985 mated pair sample was not biased due to differences in male behavior after genital coupling (Table 2-6).

Consistent with the results of 1985, just-coupled butterflies also showed opposite male-female phenotypic trends from those of the general population. Just-coupled males weighed less, were smaller, and were in worse condition than just-coupled females (Table 2-7).

The results of the tandem flight experiment provide further support that *in copula* mated pairs were an unbiased sample of the mating population. Of the 13 males tested in the tandem flight experiment, 10 (77%) were able to carry females out of the testing area. There were no differences in wing size ( $Z = -0.65$ ,  $p = 0.52$ ,  $n = 12$ ) or wing condition ( $Z = 0.81$ ,  $p = 0.42$ ,  $n = 13$ ) between successful and unsuccessful males. Furthermore, the male:female wing size ratio did not influence flight capacity ( $Z = 0.9$ ,  $p = 0.36$ ,  $n = 12$ ). The small sample size may have prevented detection of differences in male tandem flight capacity.

#### Comparisons of Butterflies Collected at Various Points During Ground-phase Precopulatory Interactions, 1986

Mated females did not differ in wing size or wing condition from females involved in copulation attempts or from females that had just-coupled, but they weighed significantly more. This difference could be a result of the weight of the just-transferred spermatophore. However, mated males, weighed after transferring the weight of a fresh spermatophore, did not weigh less than those attempting copulation or those that had just coupled (Table 2-6).

Males and females that had just coupled did not differ from those in the attempted copulation sample (Table 2-6). Because males and females in attempted copulation and just-coupled categories also

did not differ from mated pairs of that season, these sample categories were combined to form a sexually active sample and compared to the general population of 1986 (Table 2-6). Mated females were excluded from the sexually active sample for analysis of wet weight to avoid biasing the sample by the weight of just-transferred spermatophores. As in 1985, sexually active females were heavier, had longer wings, and were in better condition than females in the general population (Table 2-6). Again, as in 1985, sexually active males weighed less, were smaller, and were in worse condition than males in the general population (Table 2-6).

Females abandoned by males during ground-phase pre-copulatory interactions did not differ from those that successfully coupled with males in wet weight ( $Z = 0.80$ ,  $p = 0.43$ ), wing length ( $Z = -0.77$ ,  $p = 0.44$ ), or wing condition ( $Z = 1.59$ ,  $p = 0.11$ ) (Table 2-6). However, as determined by abdominal palpation, 90% ( $n = 53$ ) of abandoned females had mated while only 43% ( $n = 21$ ) of females that had just coupled had detectable spermatophores in the bursa copulatrix ( $X^2 = 13.58$ ,  $p = 0.0001$ ,  $df = 1$ ,  $n = 74$ ). Due to the small sample size, males left by females during ground-phase interactions were not compared to males that successfully coupled (Table 2-7).

Males attempting copulation with other males were smaller and in poorer condition than males being pursued by other males, but they did not differ in wet weight (Table 2-7). Males that took down other males did not differ from mated males in wing length or wing condition ( $Z = 1.56$ ,  $p = 0.12$ ;  $Z = -1.60$ ,  $p = 0.11$ , respectively), but they did weigh more ( $Z = 2.78$ ,  $p = 0.005$ ).

### Discussion

Monarch butterflies that mate at overwintering sites in Mexico are a non-random subset of the overwintering population. Small, light-weight males in poor condition were found mating with large, heavy females in good condition. Large males and small females were not represented in mated pairs in proportions expected from the general population if mating were random. This is contrary to my prediction of positive assortative mating, i.e., that large butterflies should pair preferentially. Although there was a slight negative association between mated male and female wing condition late in the breeding period, I found no evidence of negative assortative pairing with respect to wet weight or wing length; i.e., these characters were not correlated within mated pairs.

The magnitude of wet weight difference between mated and non-mated females is greater than that accounted for by spermatophore weight. Females in attempted copulation and just-coupled samples were significantly heavier than those in the general population, even though they had not yet received a spermatophore.

Wet weight and wing length were positively correlated with wing condition in both the general overwintering and mating populations. Because both wet weight and wing length reflect body size, it is not surprising that these two measures covary. That superior wing condition was associated with heavier wet weight and larger wing size was not expected. This finding suggests one of the following: 1) Smaller monarchs age faster under the overwintering conditions in Mexico, 2) smaller males are not older, but are in worse

condition and have lower weights because of more frequent mating efforts, or 3) the sub-set of older individuals reaching the overwintering site is more likely to be small. These influences are not mutually exclusive. The association between size and condition in both the general overwintering and mating population, in both sexes (this study) and especially very early on in the overwintering season (Alonso-M. 1996) support the third alternative.

The phenotypic character of the mating population changed through the mating period in contrast to that of the general overwintering population. Early breeding males weighed less and were in worse condition than those breeding later, but they did not differ in wing length, suggesting that early breeders may be a physiologically distinct group of small males. Early-breeding females were larger and weighed more than those breeding later in the breeding period, while they did not differ in wing condition. Whether the decrease in the mean wet weight and wing size of breeding females was due to larger females leaving the overwintering site after mating is not known (see Calvert and Lawton 1993). However, these characters did not change in females sampled from the general over-wintering population with progression of the breeding period, as would be expected if large, heavy females left the area after mating. It is also unlikely that this change in the phenotypic character of breeding females was because large, heavy females were more receptive to mating attempts earlier in the season because males also took down large, heavy males. Males may resort to mating with smaller, less weighty females later in the breeding season to avoid sperm competition (see below).

An absence of differences in size or wing condition among males or females in attempted copulation, just-coupled, and mated pair samples suggests 4 points. First, only small males capture females in aerial takedown. Whether large males in good condition do not attempt to mate or are less competent at aerial takedown awaits further study. Possible advantages of small size in male Dipterans that mate on the wing are suggested by McLachlan and Allen (1987). Marshall (1988) found that lighter-weight (but not smaller-winged) males had an advantage in aerial intraspecific competition in a Lepidopteran and attributed the advantage to lower wing loading. Second, males takedown potential mates differentially, capturing large females in good condition. Third, females do not, or cannot, reject ground-phase mating attempts by males based on male size or wing condition. Fourth, although males apparently abandon some females during ground-phase courtship, this abandonment is not based on female size or wing condition.

Abandoned females were more likely to have recently mated than those successfully paired by males, suggesting that males may discriminate against recently mated females during ground-phase pre-copulatory interactions. Oberhauser (1988) found that virgin females were more likely to be mated in caged conditions than non-virgin females. She also found that females that mated with virgins remated later than those that mated with non-virgins (Oberhauser 1989, in press). Oberhauser attributes these difference to female reluctance. My observations suggest an alternative hypothesis: males may reject non-virgin females at overwintering sites in Mexico.



Assuming fresh spermatophores originated from *in copula* males, monarchs mating at Mexican overwintering sites showed a high rate (90%) of successful spermatophore transfer. In agreement with Drummond (1984), these data suggest that success in spermatophore transfer is generally high in monarchs under field conditions.

### Female Perspectives

Pliske (1975), studying a summer breeding monarch population in southern Florida, suggested that female monarch butterflies exercise indirect mate choice by evading copulation attempts, thereby permitting only physically strong males to mate. Although overwintering females did apparently resist copulation attempts by males during ground-phase courtship, evasion seems limited as a means of female mate choice because 1) males that took down females in aerial pursuit were small and worn, and presumably should not be favored by females seeking material benefits; 2) females only rarely successfully evaded copulation attempts by these males during ground-phase interactions (pers. obs.); and 3) males that successfully coupled with females did not differ in the phenotypic characters measured from those attempting copulation. Even though males were more likely to abandon mated than unmated females during ground-phase precopulatory interactions, the consistency of female behavior and the general inability of males to evade mating attempts by other males (pers. obs.) suggest that males, and not females, were in control.

Aerial takedown also appears more likely to be under male than female control since female needs for sperm and male-provided nutrients cannot account for the absence of small females in poor condition in ground-phase precopulatory samples. Evidence that males captured by other males in aerial takedown were, like females, large and in good condition also suggests that differences in female behavioral receptivity do not account for the outcome of male aerial takedown. Although female monarchs begin gonad development later in the overwintering season than males (Herman 1985), there were no obvious differences in female pre-copulatory behavior through the breeding period. Actively flying females available for mating were of similar size, weight, and condition as clustered females (see Table 2-2). Furthermore, because gonad development and aging are associated, possible female sexual receptivity differences seem especially unlikely to account for the bias toward large females in good condition in mated pairs.

A lack of female control in mate pair formation at Mexican overwintering sites and apparent male abandonment of recently mated females support the hypothesis (Gwynne, 1984) that male investments in large nutritious spermatophores are likely to have developed through direct male reproductive benefits (as suggested by Herman 1981) and/or sperm competition avoidance rather than precopulatory choice by females.

Oberhauser (1989, 1992) found that females remated sooner in the laboratory when they had previously received smaller spermatophores, but her study did not address whether this resulted from female refusal to mate or male avoidance of sperm competition.

This study supports an alternative, non-exclusive, hypothesis to female refusal. Males may reject recently mated females to avoid sperm competition. Last male sperm precedence should generally favor male mating attempts with non-virgins. However, in Mexico, unlike at summer breeding sites, the long distance remigration required before oviposition should decrease the relative advantage of the last male to mate due to increased sperm mixing with time (Oberhauser, pers. comm.). Furthermore, even with last male advantage in sperm precedence, males may be selected to avoid non-virgins if spermatophores are costly and virgin females are readily available; as is the case at overwintering, but not summer breeding sites.

Although precopulatory female choice was not supported in this study, females may still influence which male fathers the offspring during copulation or postcopulation (Thornhill and Alcock 1983, Drummond 1984, Eberhard 1996). In this study, males that successfully transferred spermatophores weighed more and were in better condition (shown to produce larger spermatophores: Oberhauser 1988) than unsuccessful males, but they did not differ in wing length. Success in obtaining a spermatophore did not reflect female phenotype. Females terminating copulation before the complete spermatophore is transferred may gain nutrients passed into the spermatophore before the sperm (see Drummond 1984), however male lepidopterans are more likely in control of ending copulation due to their holding females with genital claspers (Wickman 1985). Even if sperm are transferred, females may be able to fertilize all or most of their eggs, via last male reproductive

advantage, by males that successfully remigrate and possibly compete at oviposition sites (see Zalucki 1993).

### Postulated Alternate Male Reproductive Behaviors

Because in this study wing condition was positively correlated with wing size (assuming wing condition reflects physiological age rather than distance traveled (see Herman 1985)), it is not clear whether initiation of sexual activity by males is size- and/or age-dependent. Variation in the initiation of sexual activity associated with male age or physiological condition could account for the observed bias toward small, light-weight, worn males in mating samples. This variation in the initiation of sexual activity, nested within the timing of colony breakup and remigration, could result in two functionally discontinuous conditional male reproductive behaviors; smaller (less weighty, more worn) males mate primarily before migration, whereas larger (heavier, less worn) males mate primarily during or after remigration.

Whether possible alternate male reproductive behaviors are the result of sexual selection cannot be determined from this study, but it is likely that males showing these alternate mating behaviors incur different costs and reproductive benefits. Small males are at a disadvantage in remigration because larger wings are advantageous in soaring, which is thought to be the primary means of migration in monarchs (Gibo and Pallett 1979). If lower wet weight reflects lower fat content, light-weight males may have little chance of successful remigration, especially if followed by intraspecific competition at

oviposition sites. Beall (1946) found that monarchs that were washed ashore on Lake Erie during the autumn migration had both lower lean weight (the weight of water and fat-free material) and significantly shorter wings than a group of clustered monarchs from beside the lake. Wing wear is also likely to hinder worn butterflies during the long remigration. Wing scale loss is an important contributor to wing wear. Because monarch wing scales contain significant concentrations of defensive cardiac glycosides (Brower and Glazier 1975, Nishio 1980, Brower et al. 1988) and probably also pyrrolizidine alkaloids (Brown 1984, Kelly et al. 1987), males in good condition may be better protected from potential predators during remigration (Brower 1985).

Males that mate at overwintering sites are ensured of at least a possibility of fathering some offspring without the need to incur costs of remigration, mate-searching, or possible male competition at oviposition sites. They may increase their reproductive success directly through nutrient contributions used for egg production and, indirectly, by providing their mates with resources (Boggs and Gilbert 1979, Wells et al. 1993) for speedy remigration and early oviposition. Such early oviposition may give larvae competitive advantages, increasing the reproductive value of each offspring produced (Waltz and Wolf 1984).

In contrast, assuming last male sperm precedence, males that are successful in migration and intraspecific competition at oviposition sites will be the likely winners in sperm competition. Males that mate at oviposition sites after migration may even

fertilize eggs produced from nutrients supplied by the males that mated at overwintering colonies.

#### Male Mate Discrimination at Mexican Overwintering Sites

Males that mate at overwintering sites are probably limited in their immediate opportunity for polygyny since time is needed to produce successive resource-rich spermatophores (Oberhauser 1988), especially if spermatophore size is positively related to reproductive success. Male resource investments may also lower future mating opportunities by decreasing the male's longevity (e.g., Boggs 1981, Shapiro 1982) or reducing his chances of remigration. Such immediate and future constraints on male promiscuity increase the benefits of pairing with large, healthy females at overwintering sites (Drummond 1984) as was found in this study. Whether or not this pairing represents active male mate choice (*sensu* Parker 1983) will be difficult to determine in the field.

Males mating at overwintering sites should also show mechanisms of sperm competition avoidance even if last male reproductive advantage occurs, if the degree of sperm mixing increases with time between mating and oviposition (Boggs 1981). Although male abandonment of recently mated females was supported in this study, a possible mechanism for this discrimination was not investigated. Male monarchs have been shown to transfer substances that could further reduce sperm competition by stimulating gonad development (Herman and Barker 1977, Herman 1985, Herman et al. 1989), speeding vitellogenesis (Herman and

Barker 1977), and possibly stimulating female movement from the area (Zalucki 1993).

### Conclusions

In this study I have shown that small, worn males mate at Mexican overwintering sites and these males pair with large females in good condition. The male takedown strategy apparently precludes precopulatory female choice. Such a non-random mating pattern does not necessarily imply that differential reproductive advantages are in effect, or that these mating patterns are the result of sexual selection. The evolutionary implications of such a non-random mating system await future work.

Table 2-1. Sample sizes of the general non-mating population, 1985, listed by sampling category and date. Sex ratio (% males) are from larger collections from which individuals were sub-sampled for obtaining wet weight, wing length and wing condition data ( \* = sex ratio not taken).

Sample Category											
Flying			Drinking			Clustered			Nectaring		
Date	n	% Males	Date	n	% Males	Date	n	% Males	Date	n	% Males
19-Feb	107	39	20-Feb	24	38	13-Feb	103	50	16-Feb	40	53
26-Feb	24	55	4-Mar	36	19	19-Feb	40	47	24-Feb	100	35
5-Mar	15	28	9-Mar	15	43	2-Mar	25	28	4-Mar	103	32
11-Mar	25	37	11-Mar	10	*	10-Mar	25	36	15-Mar	26	18
19-Mar	25	41	17-Mar	25	10	11-Mar	25	32	16-Mar	15	7
25-Mar	7	*	25-Mar	14	*	16-Mar	25	38	17-Mar	10	*
						23-Mar	15	30	23-Mar	25	28
Total	203			124			258			319	



Table 2-2. Comparisons of wet weight (mg), forewing length (mm), and wing condition (no units; see text) of males and females among general population sampling categories in 1985 (Kruskal-Wallis test, Chi-square approximation,  $df = 3$ ) for early (before 5 March) and late (on or after 5 March) samples.

Measure	Flying			Sample Category									X <sup>2</sup>	p
	n	Drinking		Clustered			Nectaring							
		Mean	SD	n	Mean	SD	n	Mean	SD					
Early (before 5 March)														
Wet Weight														
Males	51	436	65	16	425	65	81	471	58	89	445	48	13.01	0.005
Females	80	402	76	44	402	61	86	449	70	154	382	54	45.69	0.0001
Wing Length														
Males	48	52.2	2.3	16	51.5	2.2	81	52.3	2.0	88	52.4	1.9	3.77	0.29
Females	80	51.5	2.3	44	51.9	1.6	85	52.0	1.9	154	51.3	2.5	5.43	0.14
Wing Condition														
Males	51	1.6	0.7	16	1.6	0.5	82	1.6	0.6	89	1.7	0.8	2.50	0.48
Females	80	1.4	0.7	44	1.6	0.7	86	1.6	0.7	154	1.6	0.7	5.37	0.15
Late (on or after 5 March)														
Wet Weight														
Males	15	471	49	15	466	54	25	452	58	18	430	56	5.72	0.13
Females	57	427	60	49	413	71	65	393	62	58	362	61	26.97	0.0001
Wing Length														
Males	15	53.2	2.3	15	52.4	2.2	25	52.6	1.7	18	52.3	1.7	2.14	0.54
Females	57	52.3	1.5	49	51.7	2.3	65	51.8	1.9	58	51.5	2.2	3.47	0.32
Wing Condition														
Males	15	1.8	0.7	15	1.7	0.7	25	1.5	0.7	18	1.7	0.7	2.11	0.55
Females	57	1.5	0.6	49	1.9	0.6	65	1.6	0.6	58	1.8	0.6	11.61	0.009

Table 2-3. Butterflies in each of the wing condition categories (good, medium, poor) compared with respect to wet weights (mg) and forewing lengths (mm) for both the general population and mated pair samples in 1985 using Kruskal-Wallis tests (Chi-square approximations,  $df = 2$ ). All  $p$  values  $< 0.006$ .

Wing Condition	Wet Weight			Wing Length		
	General Population		Mated Pairs	General Population		Mated Pairs
	n	Mean	SD	n	Mean	SD
Males						
Good	149	468	53	147	465	59
Medium	126	443	56	194	432	69
Poor	35	411	58	94	385	78
$\chi^2$		30.7			66.7	
					12.2	26.6
Females						
Good	294	414	68	332	478	65
Medium	240	397	66	103	452	62
Poor	59	364	65	7	448	35
$\chi^2$		27.7			13.9	
					13.9	10.3

Table 2-4. Wet weight (mg) and forewing length (mm) comparisons for males and females in the general population and mated pairs (11 February - 25 March 1985) using Wilcoxon two-sample rank-sum tests (normal approximation; continuity correction of 0.5). ( $p < 0.001$ , except \* = not significant at  $p = 0.05$ ).

General Population				Mated Pairs			
	n	Mean	SD	n	Mean	SD	Z
Wet Weight							
Males	310	451	58	435	433	74	3.13
Females	593	402	68	442	472	64	-15
Z		10.9			-7.6		
Forewing Length							
Males	306	52.3	2	446	51.4	2.6	5.14
Females	592	51.7	2.1	451	52.3	1.8	-4.5
Z		4.64			-5.1		
Wing Condition							
Males	311	1.6	0.7	449	1.9	0.7	4.48
Females	593	1.6	0.7	451	1.3	0.5	-8.8
Z		0.59 *			-13		

Table 2-5. Wet weight (mg) and forewing length (mm) comparisons for males and females in the general population and mated pairs for early- (before 5 March) and late-season (on or after 5 March) 1985 samples using Wilcoxon two-sample rank-sum tests (normal approximation; continuity correction of 0.5). (\*  $p < 0.0005$ ; NS = not significant at  $p = 0.05$ ).

	General Population			Mated Pairs				
	n	Mean	SD	n	Mean	SD	Z	p
Early (before 5 March)								
Wet Weight								
Males	237	451	58	122	416	71	-4.20	*
Females	364	405	69	122	490	61	11.00	*
Forewing Length								
Males	233	52.3	2.0	124	51.1	2.7	-3.94	*
Females	363	51.6	2.2	125	52.7	1.6	5.24	*
Wing Condition								
Males	238	1.6	0.7	125	2.1	0.7	6.04	*
Females	364	1.6	0.7	125	1.3	0.5	-3.99	*
Late (on or after 5 March)								
Wet Weight								
Males	73	453	56	313	440	74	1.36	NS
Females	229	398	67	320	465	65	-10.77	*
Forewing Length								
Males	73	52.6	1.9	322	51.5	2.5	3.63	*
Females	229	51.8	2.0	326	52.2	1.9	-1.53	NS
Wing Condition								
Males	73	1.7	0.7	324	1.8	0.7	-1.27	NS
Females	229	1.7	0.6	326	1.3	0.5	8.75	*

Table 2-6. Male and female wet weight (mg), forewing length (mm), and wing condition (no units; see text) are compared among various sampling categories for 1986 data using Wilcoxon two-sample rank-sum tests (normal approximation; continuity correction of 0.5). Sexually active sample is combined just-coupled, attempted copulation, and mated pair samples from the 1986 overwintering period. The mated pair sample was excluded from the combined sample for comparison of female wet weight; see text.

Measure	Compared Sample Categories									
	Just-coupled vs			Attempted copul. vs			Sexually active vs			
	Mated pairs			Mated pairs			Just-coupled			General population
	Z	p		Z	p		Z	p	Z	p
Wet Weight										
Males	-0.13	0.90		-0.90	0.37		-0.54	0.59	-4.8	0.0001
Females	-2.11	0.03		-3.22	0.001		1.02	0.31	10.5	0.0001
Forewing Length										
Males	-1.38	0.17		0.34	0.74		-1.28	0.20	-6.3	0.0001
Females	-1.42	0.15		0.08	0.93		-1.23	0.22	3.5	0.0004
Wing Condition										
Males	-1.57	0.12		-1.88	0.06		-0.15	0.88	-3.2	0.002
Females	0.42	0.68		0.60	0.55		-0.20	0.84	8.3	0.0001

Table 2-7. Comparison of male versus female wet weights (mg), forewing lengths (mm), and wing conditions (no units; see text) from five sampling categories from 1 March to 25 March 1986 using Wilcoxon two-sample rank-sum tests (normal approximation). (\*  $p < 0.05$ ; \*\*  $p < 0.005$ ; NS = not significant at  $p = 0.05$ ).

	Males			Females				
	n	Mean	SD	n	Mean	SD	Z	p
Wet Weight								
Mated Pairs	65	427	73	67	506	77	-5.45	**
Just-Coupled	51	423	107	63	478	67	-2.81	**
Attempted Copulation	66	436	75	66	464	62	-1.92	(NS)
Homocourtship	47	464	64	50	485	66	-1.35	(NS)
Abandoned	5	433	54	23	466	67	0.95	(NS)
General Population	429	463	61	452	418	59	10.61	**
Forewing Length								
Mated Pairs	57	51.6	2.6	67	52.5	1.6	-1.74	(NS)
Just-Coupled	55	50.8	3.3	75	53.0	1.8	-3.87	**
Attempted Copulation	59	51.6	2.4	64	52.5	2.0	2.20	*
Homocourtship	40	52.6	1.7	50	53.5	1.6	-2.47	*
Abandoned	5	52.3	2.8	23	52.8	1.7	0.09	(NS)
General Population	391	52.9	1.8	435	52.1	1.9	6.07	**
Wing Condition								
Mated Pairs	66	1.9	0.7	67	1.4	0.6	-4.85	**
Just-Coupled	65	2.1	0.8	77	1.3	0.5	6.32	**
Attempted Copulation	66	2.2	0.7	66	1.3	0.5	-6.83	**
Homocourtship	48	2.1	0.6	51	1.7	0.6	-3.69	**
Abandoned	5	1.4	0.6	23	1.5	0.5	0.28	(NS)
General Population	429	1.9	0.7	452	1.8	0.7	1.91	(NS)

*Danaus plexippus* (L), Overwintering Colony, Mexico 1985

Right Forewing Length Range

38.5 - 58.0 mm



Figure 2-1. Examples of the 2 extremes of wing size variation among Mexico overwintering monarch butterflies in 1985. Females were generally smaller than males (see text). However, both the largest and the smallest individuals collected were females. Relatively small changes in forewing length result in relatively large changes in overall wing size.

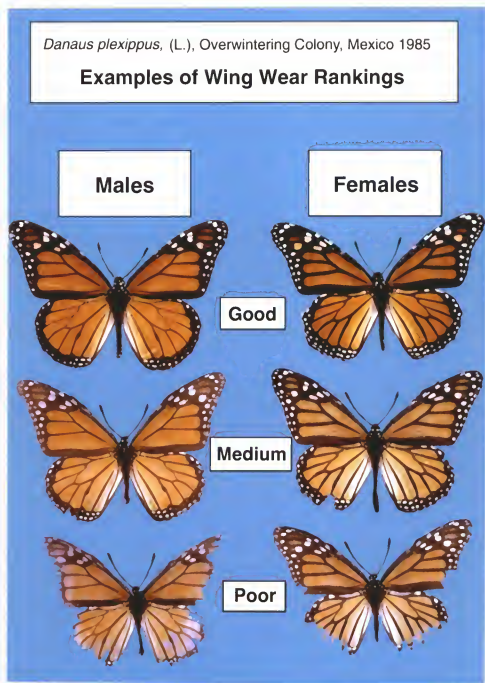


Figure 2-2. Representative examples of monarch wing condition ranking categories: good, medium and poor. Butterflies on the right are females, those on the left are males. Females are distinguishable from males by the absence of the black scent organs on the hind wings and the relatively wider wing cell veins. Degree of scale fading, scale loss, scratches, and tattering (fraying) of wing margins were used to rank wing condition. Wing condition was used as estimate approximate physical age (see text).



### CHAPTER 3

## PROXIMATE CAUSATION OF SMALL, WORN MALE MATING ADVANTAGE IN OVERWINTERING MONARCHS

### Introduction

Each autumn an extensive migration brings nearly the entire North American population of monarch butterflies, *Danaus plexippus* (L.), into dense overwintering aggregations in central Mexico for several months. These aggregations are extremely far removed from larval resources in space and time. Most of the winter is generally spent in reproductive diapause (Herman et al. 1989). The following spring, an exponentially increasing mating event occurs just before remigration from these sites (see Chapter 2).

A striking aspect of the monarch mating system is the male takedown strategy in which males pursue and attempt to capture females. Aerial pursuit is obscured at the overwintering site by the extreme density of butterflies in flight in the colony area where mating primarily takes place (Leong et al. 1995, Chapter 2). However, my observations in Mexico and those of workers studying California monarchs (Hill et al. 1976, Leong et al. 1995, pers. obs.) suggest that overwintering pre-copulatory interactions are superficially indistinguishable from summer breeding monarchs . Unlike the closely related queen butterfly (*Danaus gilippus berenice* (Cramer), Brower et al. 1965), pheromone communication is apparently not required for mating success (Pliske 1975). Also

unlike queen butterflies, and perhaps related to the reduced importance of the much smaller hairpencils of male monarchs, females evade male takedown attempts and resist ground phase mating attempts (Pliske 1975, Boppré 1993, pers. obs.). The extreme density of adults at overwintering sites may have acted as a selective agent against pheromone use on monarchs.

In mating systems in which population densities are very high and males are unable to defend females or resources needed by females, a large male mating advantage is expected due to the increased ability of large males to capture and overcome females (Thornhill 1980, Partridge et al. 1987, Taylor and Kekic 1988). However, the relative advantage of male size depends on how they capture females, and the degree of female cooperation and her ability to resist capture and mating attempts. Small males may sometimes be at an advantage due to improved aerobic ability (see McLachlan and Allen 1987, McLachlan and Neems 1995).

A competitive advantage of large males is not easily separated from a female mating preference for large males that is expected on grounds of either material or genetic benefits (Rutowski 1982). For example, Maynard Smith (1956) suggested that female evasion during courtship might function to test the male's 'athletic ability' in *Drosophila subobscura*. Using this same reasoning, Pliske (1975) suggested that female monarchs evade males during aerial chases to assure that only strong males mate.

Female monarchs are expected to benefit from mating with large, good condition males because males make large nutrient investments in spermatophores that females incorporate into their

somatic tissues and utilize in egg production (Boggs and Watt 1981, Wells et al. 1993, Oberhauser 1988, 1989). Furthermore, female nutrient need is expected to be high due to the long overwintering period in which stored adult resources are depleted for somatic maintenance, and because a long distance spring re-migration is necessary for females to reach their oviposition sites (Herman 1981, Tuskes and Brower 1978, Brower 1985, Wells et al. 1990, Wells et al. 1993, Oberhauser and Hampton 1995). Finally, the high population densities in the Mexican overwintering colony are expected to provide a heightened opportunity to exercise choice (Eshel 1979, Janetos 1980, Wade 1980, Thornhill and Alcock 1983; but see O'Donald 1978, McLain 1982, Arnqvist 1992).

I hypothesized (Chapter 2) that these unique aspects of monarch natural history should lead to a large male mating advantage during this mating period both through male-male competition and female choice. These selective pressures should act in concert and their relative impact of male mating success should depend on the relative proportion of females available for takedown, the length of the mating event, and the degree of female control in mating (Thornhill and Alcock 1983).

Contrary to my predictions, I found that mated males were light weight, had small wings, and were in poor condition. The observed small, worn male mating advantage was not due to a sampling bias. Males carry females in tandem flight to a site where they remain *in copula*, usually overnight (Svärd and Wiklund 1988b). If large, good condition males carried females outside of the colony area, they would not be represented in mated pairs which

were collected from the colony area. However, a test of male ability to carry females in tandem flight, and an absence of phenotypic differences between *in copula* males captured before versus after tandem flight suggest that the small male advantage occurred before tandem flight. Furthermore, the small, worn male advantage was not a result of female discrimination during the ground phase of pre-copulatory interactions. Males that took down females in aerial pursuit did not differ from those that successfully coupled.

Assuming wing condition reflects age (see Herman 1985), both size and age may influence male propensity or ability to takedown females. Small size was associated with poor wing condition in both mating and general population samples of both males and females. Males mating during the first half of the approximately 6 week mating period differed from those mating in the latter half. Early maters were not only smaller, but light in weight and more worn compared to males in the general population. Males mating during the second half of the mating period differed from the general population only in being smaller in size.

In summary, I found that small, worn males capture females in aerial takedown (Chapter 2), but whether this was a result of a small male advantage in aerial pursuit, a size, and/or condition dependent difference in male activity or motivation to mate, or a combination of these was not investigated. Assessment of male takedown ability under field conditions was not possible due to the extreme densities of monarchs in flight. In this study I use 3 methods to test 2 proximate hypotheses proposed to account for why large, pristine males were underrepresented in mated pairs.

Hypothesis 1: Large, Good Condition Males Do Not Attempt to Mate

I used 2 methods to determine whether large, good condition males were attempting to mate. First, if only small, worn males become active and leave quiescent roosting clusters, then a phenotypic difference unrelated to male takedown ability could account for the observed small male mating advantage. I determined if the sub-set of active males available to mate included large, good condition males by comparing male size, wet weight and wing condition among various activity categories.

Alternatively, if only males that takedown females have developed reproductive tracts, then size and/or age related differences in male reproductive state could explain the observed male mating advantage. The cessation of monarch reproductive diapause is associated with increasing levels of juvenile hormone which causes increased reproductive tract tissue weights and initiation of sexual behavior (references in Herman 1985, 1993). I compared relative tubular gland wet weight of mated males with those collected from the general overwintering population during the mass mating period in 1988. Because overwintering mating males were smaller and, at least in early maters, in worse condition, I also tested for differences in reproductive tract tissue weights with respect to wing size and wing condition. Testis weight does not change in response to juvenile hormone levels (Herman et al. 1981), and testis wet weight does not increase during the mating event in

Mexico overwintering males (Herman et al. 1989). I therefore used testis weight as a control.

### Hypothesis 2: Small Male Takedown Advantage in Aerial Pursuit

Small male aerodynamic advantage based on size (McLachlan and Allen 1987, six species of Diptera) and weight (Marshall 1988, two species of moths) has been suggested for insects that mate on the wing. Steele and Partridge (1988) also showed a small size advantage in *Drosophila subobscura* male ability to track moving, but not flying, females.

If there is a small male aerodynamic advantage, I predicted that small males would more likely be the first to takedown females in paired tests with large males. In a semi-natural environment, I tested these predictions using laboratory reared summer generation monarchs. By controlling for male age and wing condition differences, I could separate out these possibly confounding influences from size on male motivation and/or ability to takedown females. Single small and large male pairs were tested in a large flight cage containing free flying females. Because any advantage related to male ability to keep up with and track a female during aerial pursuit would be eliminated in a small cage that constrains the flight component of precopulatory interactions, the hypothesized size advantage would be absent when the same 2 males are tested for copulatory success in a small cage.

As indicated above, hypotheses 1 and 2 are not mutually exclusive. However, these tests should allow me to determine the

importance of the male's reproductive state and flight ability in defining attributes of male mating success, and whether these factors interact.

### Methods

#### Hypothesis 1: Large, Good Condition Males Do Not Attempt to Mate

##### 1985 Male activity versus phenotype comparisons

I compared male wing length, wet weight and wing condition among 1) clustered, 2) flying, 3) drinking, 4) nectaring, and 5) *in copula* activity categories during the mass mating period in 1985. Sampling methods are reported in Chapter 2. Multiple samples collected from 10 February through 25 March were combined from each activity category. These combined samples were compared with regard to male size, wet weight and wing condition.

##### 1988 Male reproductive tract development versus mating status

In 1988 mating activity became common on 19 February, increased dramatically in mid-March, and remained high until the butterflies dispersed abruptly on the 22nd of March (see Chapter 2). Only about 10% of the colony remained on the following day. The early season collection was made from 25 - 26 February, the mid-season collection was made from 11 - 12 March, and the late season collection was made from 22 - 23 March. In each 2 day collection period, the clustered male sample was collected on one day and mated males were collected the alternate day. These collection dates

correspond to the beginning, middle and late phases of the mating event.

The 3 general population samples (early, middle and late season) were each taken from a single tree positioned near the center of the overwintering colony. A butterfly net attached to a long pole was used to capture a single bough cluster of quiescent roosting monarchs. From this sample of several hundred individuals, I sub-sampled 20 males. For convenience I will refer to these as non-mating males, but I have no information as to their mating history. Assuming that sexually active males return to clusters to roost at night, these samples at least potentially include sexually active males. My method of contrasting reproductive tract development between mating and non-mating males should therefore be conservative. Mated males were collected as *in copula* pairs from inside the colony area and within 3 m of the ground. Each of the 3 collections consisted of 20 males. Because mating males are generally smaller and, at least early-on, in worse condition, I could not control for differences between the mating and general population collections with respect to wet weight, wing size, or wing condition.

Clustered males, or *in copula* pairs, were stored in glassine envelopes on ice until weighed, measured, and ranked for wing condition within 24 h. Wet weight was measured to the nearest 1 mg using a Sartorius Model 1205 MP balance. Forewing length was measured to the nearest 0.5 mm along the costal margin from base to apex. Wing condition was ranked using a 5 point scale with 0.5 intervals ranging from excellent to very poor. The 5 point scale



differed from methods used in the other chapters in which I only distinguished good, medium and poor wing condition (see Figure 2-1 in Chapter 2). Rankings were based on visual assessment of scale loss, scale fading, number of scratches, and degree of wing margin tattering. Each butterfly was held up to a light source while holding the wings spread open so that both the upper and lower wing surface could be inspected. I compared each male to a set of 5 mounted monarch specimens representing each wing condition category in order to maintain ranking consistency. Because my objective was to use wing condition to estimate approximate physical age rather than distance traveled or experiences incurred, I attempted to ignore damage such as beak marks, missing wing area believed to be attributable to bird damage, or damage due to net capture or handling, such as thumb and net prints. Whole animals were then stored in liquid nitrogen until dissected, except during the trip from the field to the laboratory when they were carried on dry ice.

#### Reproductive tract dissections

Male reproductive tract dissections followed the methods of Herman (1975b), with minor modifications reported here. I re-weighed males in the laboratory just before dissection in saline solution. I first removed the entire reproductive tract from the abdomen by cutting the common duct from the aedeagus at the bulbus ejaculatorius. I then cut the testis from the vas deferens, and the common duct from the vesicula seminalis (see Figure 3-1). Reflecting the terminology of Herman (1975b), I will refer to the

entire length of the common duct as the tubular gland. This length consists predominantly of the tubular gland, but also includes the ductus ejaculatorius and bulbus ejaculatorius as described by Urquhart (1960, p. 246). The ductus ejaculatorius and bulbus ejaculatorius are not externally distinct from the tubular gland, and so were included in order to maintain sampling consistency. I removed attached fat body cells and tracheae from the testis and tubular gland using forceps, and after blotting on filter paper to absorb the saline solution, weighed each to the nearest 0.1 mg using a Mettler AK 160 electronic balance. Herman (1981) showed that the wet weight of the tubular gland is highly correlated with the dry weight. Only wet weights are reported here.

#### Lipid analysis

I randomly sampled 7 small and 7 large males from the mated and non-mating male samples for lipid analysis. Small males were defined as those with a forewing length less than or equal to 52 mm. Large males were defined as those with forewing lengths greater than or equal to 52.5 mm. These 28 males, minus the head (used for another experiment), the testis, and the tubular gland, were dried at 60 ° C for 16 h and then individually extracted for lipids in petroleum ether (Walford 1980, May 1992).

### Statistical analyses

By visual inspection my data were not normally distributed. I therefore chose to use non-parametric statistics. However, because the data were unimodal and without large deviations in symmetry, I report mean values, and standard errors for visual comparisons between and among groups. I also report frequency distributions for comparison of relative tubular gland and testis wet weights. All tests were corrected for ties when appropriate. All statistical tests were conducted using Stat View 4.01, universal version, for the Macintosh. Mann-Whitney tests are two-tailed normal approximations using the Z test statistic.

### Hypothesis 2: Small Male Takedown Advantage in Aerial Pursuit

#### Rearing

All adults used in this semi-natural experiment were reared under laboratory conditions from eggs collected either from wild caught females or from *Asclepias humistrata* plants. Both the females and the eggs were collected from a field of *A. humistrata* near the junction of SR 346 and SR 325, Alachua County, Florida, from April through May 1992. The larvae were reared singly in plastic containers with an abundant supply of *A. humistrata* leaves. All larvae were reared under a 12 h light phase. Laboratory temperatures ranged from 23.5 - 24.5 °C.

In order to obtain small and large males of the same age each day, each day I removed the food from half of the larvae that were

on the 4th day of their 5th instar development. A pilot study showed that larvae usually pupate on the following day. This method resulted in approximately 24 h of starvation. I recorded the sex, starvation category (starved versus non-starved), hatch date, pupation date, eclosion date and, 24 h after eclosion, right forewing length and wet weight.

To prevent wing wear and wing damage, after I weighed and measured the adults, I stored them individually in glassine envelopes in the laboratory at room temperature. Starting on the 3rd day after eclosion and then every other day thereafter, I fed them a 30% sugar:tap water solution until satiation. The day before testing, males were released into a large outdoor flight cage positioned adjacent to the testing flight cage containing free flying females. All tested males were virgins and at least 5 days old to ensure that they were reproductively mature (see Oberhauser 1988).

#### Tests of possible negative effects of larval starvation on adult vigor

I observed no differences in vigor between adults derived from starved and non-starved larvae. However, to assess whether temporary starvation might negatively influence adult health or behavior, I compared various aspects of larval developmental times (at 24 h intervals) between starved and non-starved larvae. These comparisons included 1) total development time (hatch to eclosion), 2) larval development time (hatch to pupation), and 3) pupal development time (pupation to eclosion). The striking result was that development times were extremely invariable and similar

between starved and non-starved groups (see Figure 3-2). I also compared the sex ratio of emerging adults, and percent immature deaths (hatch to eclosion) between starved and non-starved larval groups. Using Chi-square analyses with continuity correction, there were no differences between starved and non-starved groups ( $X^2 = 19$ ,  $df = 1$ ,  $p = 0.17$ ,  $X^2 = 0.2$ ,  $df = 1$ ,  $p = 0.65$ ; sex ratio and percent immature deaths, respectively).

I made 2 further comparisons of starved versus non-starved larvae from a pilot study conducted in 1991. These larvae were reared using the same protocol as described above for the 1992 experiment.

First, I measured mean wet weight change between either 3 or 4 consecutive feeding bouts. Starting on the 3rd day after eclosion, adults were first weighed to the nearest 0.1 mg using a Mettler AK 160 electronic balance and then fed as described above. Two days later, I again weighed the adult just before feeding. Weight changes, measured as the previous pre-feeding weight minus the present pre-feeding weight, were sometimes negative and sometimes positive since the butterflies fed until satiation between these measurements, they were stored in glassine envelopes restricting energy use, and 48 h had passed. The mean change in wet weight in either 3 to 4 successive feeding bouts was compared between starved and non-starve larvae. Larger mean wet weight changes indicate that adults were less able to regain weight by feeding; an indication of reduced vigor. I found no detrimental affects of larval starvation on adult vigor. Starved and non-starved larvae did not differ in the mean

wet weight change between feeding bouts ( $Z = 0.72$ ,  $p = 0.47$ ,  $n = 43$ ; range = 3.8 - 50 mg and 4.9 - 46 mg, respectively).

In a second test of adult vigor, I compared the crawling rates of adults that were starved versus non-starved as larvae. I compared the time, in s, that it took 1 to 4 day old adults to crawl up the inside walls of a 45 cm mailing tube. In the laboratory where the larvae were reared, a mailing tube was positioned vertically on a ring stand. The top of the tube was uncapped with a low wattage incandescent light placed immediately above the opening. The tube was lined with mosquito net to provide a surface that the butterflies could easily traverse. During each trial, the bottom cap of the tube was removed, the adult placed just inside, and the cap replaced. The total crawling time was measured from the time the bottom cap was replaced until any part of the adult emerged above the top of the mailing tube. The mean of 3 trials conducted on consecutive days was used as the measure of crawling rate, with the following exceptions. In a total of 10 trials (6 starved and 4 non-starved males) the butterfly did not begin crawling up the tube when the cap was replaced. All of these were re-tested on the same day after all other trials were run. All but 2 of these (one starved and one non-starved) immediately traversed the tube. The crawling rate of these re-trials were used in the analysis as 1 of 3 total trials. In the 2 remaining males, I used the mean of the 2 successful trials. Using male motivation and/or ability to crawl as an indicator, I found no significant affect of larval starvation on male vigor ( $Z = 0.95$ ,  $p = 0.34$ ,  $n = 42$ ; range = 22.5 - 34.9 s and 21.1 - 38.0 s for starved and non-starved males, respectively).

Apparently, the effect of starvation was to increase the relative frequency of lower weight and smaller wing length adults. The size and wet weight distributions of adult males that were starved for 24 h versus those fed continuously as larvae overlapped considerably (see Figure 3-3). However, adults temporarily starved as larvae had significantly lower wet weights, shorter wing lengths, and lower wing loadings (as estimated by  $\text{wet weight} / (\text{forewing length})^2$ ); see Calvert and Lawton 1993) compared to non-starved larvae (see Table 3-1).

### Experimental design

A single large and small male pair was first tested for takedown ability in a large outdoor flight cage in which the males could pursue females in flight. After all trials for a particular day were completed in the large flight cage, the same small-large male pairs were again tested for mating ability in a small cage with a single female. The flight component was eliminated in the second test by the confines of the small cage.

The large flight cage was an outdoor screened porch measuring 5.5 X 5.5 X 3 m, filled with nectar and host plants and containing 20 to 30 free flying females. Each small-large male pair was released simultaneously into the flight cage and allowed to move freely with the females. Females were induced to fly just before I released the males by jostling the plants upon which they periodically came to rest. Because butterfly flight activity apparently stimulated males to chase potential mates, I similarly attempted to keep a large

proportion of the females in flight at all times during each trial. I also enticed the 2 males to fly continuously by preventing them from resting on any surface upon which they alighted. After testing, each male was immediately returned to a glassine envelop and stored on ice in a cooler until all male pairs were tested for that day.

The small cages were nylon mesh cylinders hung within a second large cage adjacent to the large flight cage. The small cages varied somewhat in size, but were approximately 40 cm in diameter and 55 cm in height.

I chose each small-large male pair based on forewing length without regard to wet weight. Small males were defined as those with forewing lengths less than or equal to 52.0 mm. Large males were those with forewing lengths greater than or equal to 53.0 mm. I further required that there was at least a 1.5 mm difference in wing length between each small-large male pair tested. Both small and large males, as defined, included starved and non-starved larvae groups.

The day before each testing, single large-small male pairs were randomly chosen from those available matched for age by date of eclosion. All males to be tested on the following day, were removed from their glassine envelops and released in an outdoor flight cage adjacent to the testing cage containing the free flying females. Males that did not fly readily were not used in the experiment.

All females used in this experiment were obtained from the larval rearing protocol described above. They were virgins and at least 5 days old to insure they were sexually mature (Oberhauser 1989). Females in the large flight cage were fed just before their



initial release. They also free-fed on nectar from potted flowers, and were captured periodically and fed 30% sucrose:tap water solution to satiation. Females were kept continuously in the large cage until death or until they did not fly vigorously. Such females were removed and replaced with females from laboratory rearings. Females were assessed and counted every day before the male flight trials began. Due to changes in availability of females through time I used either 20 or 30 females in each trial. My intention was to provide enough females so that many were in flight simultaneously, reducing potential male-male competition for active females, so I assume the difference in the number of females does not influence my results.

#### Large flight cage trials

In the large flight cage, the first male to successfully takedown a female was noted as the winner. Trials were from 15 May through 3 July 1992 between 1300 and 1845 h in sunny to partly cloudy conditions. Ambient temperatures ranged from 26 °C to 37 °C. Trials lasted 20 m or until 1 of the males successfully took down a female. If neither male captured a female the trial was designated as a mis-trial, and both males were returned to the available group from which small-large male pairs were chosen for testing beginning the next day. The winner often coupled with the female by the time I could catch it. If so, I immediately separated the male-female pair so no sperm or materials could be passed to the female.

Male attempts to chase and capture females in the large cage were indistinguishable from natural chases that I observed at the Mexico overwintering site. However, the chase was sometimes interrupted when the butterflies encountered the screen walls of the cage.

### Small cage trials

After all tests in the large flight cage were finished on a particular day, each of the small-large male pairs were added to a small net cage containing a single sexually mature virgin female at least 5 days old. These females were reared in the laboratory but were not those used in the large flight cage experiment. The male that successfully coupled with the female was noted as the winner. Small cage trials lasted 24 h. The cages were checked, and mated pairs noted, that evening, again around mid-night and at dawn when pairs most often uncouple (Svärd and Wiklund 1988b). Females were dissected to make sure that only one male mated. If neither male coupled with the female, the trial was considered a mis-trial.

Only small-large male pairs in which there was a winner in both the large and the small cage were included in the analyses. Of these 27 small-large male pairs, the mean forewing length for males in the small and large size category was  $50.1 \text{ mm} \pm 0.4 \text{ SE}$  and  $54.1 \text{ mm} \pm 0.2 \text{ SE}$ , respectively. The mean wet weight at eclosion for small and large males was  $518 \text{ mg} \pm 15 \text{ SE}$  and  $645 \text{ mg} \pm 12 \text{ SE}$ , respectively.

### Statistical analyses

Because I tested the same pair of males in both the large and the small cage, the critical statistical test is to compare the combinations of winners in pairs of flight versus no flight cage treatments using a Chi-square test with cage size as a repeated measure. I therefore used SAS Categorical Modeling (SAS 1985) to compare paired tests of male mating advantage in small versus large cages. This analysis tests whether cage type, i.e. the presence or absence of the opportunity for aerial takedown, influenced the outcome of whether the large or small male won.

### Results

#### Hypothesis 1: Large, Good Condition Males Do Not Attempt to Mate

##### 1985 Male activity versus phenotype comparisons

Using Kruskal-Wallis tests, male size (wing length), wet weight, and age (wing condition) varied among activity categories ( $H = 22.41$ ,  $28.64$ , and  $31.41$  respectively;  $df = 4$ ,  $p < 0.0001$  in all cases). These differences are summarized in Figure 3-4. Multiple comparisons were not conducted, but by inspection it can be seen that 1) large, good condition males are available in the active categories, including flying, drinking and nectaring samples, and 2) mated males most differed from the other activity groups in size and condition, consistent with the results of Chapter 2. Only wet weight is apparently greater in clustered versus active male samples. These

comparisons suggest that there is no measured phenotypic bias in active versus inactive samples that could account for the small male mating advantage observed in 1985. Mating males are a distinct sub-set of active males.

#### 1988 Male reproductive tract development versus mating status

Using Spearman rank-order correlation, both testis wet weight and tubular gland wet weight were weakly but positively correlated with male whole body wet weight ( $r_s = 0.22$ ,  $Z = 2.44$ ,  $p = 0.02$ ,  $n = 120$ ). Because I could not control for size differences between mating and non-mating males, I chose to report organ weights as relative weights. Relative wet weight of both the testis and the tubular gland are defined as the organ's wet weight divided by the male weight taken at the time of dissection multiplied by 100.

Combining early, middle and late season samples, differences between mating and non-mating male phenotypes in this study were consistent with my findings from 1985 (Chapter 2). As measured by wing length, mating males were smaller than non-mating males ( $Z = 2.21$ ,  $p = 0.03$ ,  $n = 120$ ). Using wing condition as an indication of age, they were also older ( $Z = 4.15$ ,  $p < 0.0001$ ,  $n = 120$ ). The mating males were also very nearly significantly lower in wet weight ( $Z = 1.90$ ,  $p = 0.06$ ,  $n = 120$ ).

Figure 3-5 summarizes the overall differences in relative tubular gland and testis wet weights between mating and non-mating males. Mating male relative tubular gland wet weight differed from males in the general population, but in the opposite

direction as predicted. Mated males had lower tubular gland weights than non-mated males ( $Z = 4.26$ ,  $p < 0.0001$ ,  $n = 120$ ). Relative testis wet weight did not differ between mating and non-mating males ( $Z = 0.87$ ,  $p = 0.39$ ,  $n = 120$ ).

The above trends, based on combined early, middle and late mating event samples, obscure some differences between mating and non-mating males and changes in these populations through time. Male wet weight, wing length, wing condition, relative testis wet weight, and relative tubular gland weight comparisons in early and late season samples are shown in Table 3-2. *In copula* males showed similar trends in phenotype and reproductive tract development in early and late season samples. However, the general population differed over time. In the early mating season, males in the general population were larger and in better condition, but did not differ from mating males in measured reproductive tract development. At the end of the mating period, males in the general population came to resemble the mating male population in size and wet weight, but remained in better condition. In contrast to the absence of differences observed in the early mating period, late overwintering males collected from clusters had both larger relative testis and tubular gland wet weights compared to mating males (see Table 3-1).

As expected if more and more males in the general population were breaking diapause through time, their relative tubular gland wet weight increased between the early and late mating period ( $Z = 4.4$ ,  $p < 0.0001$ ,  $n = 39$ , see Figure 3-1). The increase in testis wet weight ( $Z = 3.18$ ,  $p = 0.002$ ,  $n = 39$ ) is also consistent with trends on post-eclosion testis development of summer monarchs (Herman et al.

1981). Unlike males in the general population, there was no difference in relative testis or tubular gland wet weight between early and late season mated males ( $Z = 0.57$ ,  $p = 0.57$ ,  $n = 41$ ;  $Z = 0.99$ ,  $p = 0.32$ ,  $n = 41$ , respectively).

There was no significant trend between male size and relative tubular gland wet weight in mated or non-mated males ( $Z = 0.94$ ,  $p = 0.34$ ,  $n = 60$ ,  $Z = -1.11$ ,  $p = 0.27$ ,  $n = 60$ , respectively). However, male size versus relative tubular gland wet weight showed interesting trends in mated males. First, relative tubular gland weight was consistently very low small mated males, while larger males showed higher and more variable weights similar to those found in the general overwintering population (see Figure 3-6). Another striking result was that a large proportion of mated males had very low tubular gland weights regardless of their size, while tubular gland weights of males in the general population were less variable but generally higher than mated males. The percent of mated and non-mated males with relative tubular gland weights above 3.0 (approximately the upper 2/3 of the range of relative weights) was 13% versus 48% respectively (see Figure 3-6). This difference between mated and non-mated males is also seen in Figure 3-5 showing the overall distribution of relative tubular gland weights in mated versus general population males.

As expected if older males had more developed reproductive tracts, poorer male wing condition was associated with larger relative tubular gland wet weight in the general population ( $r_s = 0.56$ ,  $Z = 4.27$ ,  $p < 0.0001$ ,  $n = 60$ ). However, there was no such association in mated males ( $Z = 1.27$ ,  $p = 0.21$ ,  $n = 60$ ). Together these results

suggest that reproductive tract development may be associated with aging, or *vice versa*, but this trend does not explain male mating status.

Relative testis wet weight was negatively correlated with male size in both mated and non-mated males; although significant only in non-mating males. However, male size accounted for only a small percentage of the variation in relative testis wet weight ( $r_s = -0.25$ ,  $Z = -1.90$ ,  $p = 0.06$ ,  $n = 60$ ,  $r_s = -0.37$ ,  $Z = -2.83$ ,  $p = 0.005$ ,  $n = 60$ ; mated and non-mated males, respectively).

Relative testis wet weight and wing condition were also correlated in mated, but not non-mated males ( $r_s = -0.34$ ,  $Z = 2.58$ ,  $p = 0.01$ ,  $n = 60$ ,  $Z = 1.37$ ,  $p = 0.17$ ,  $n = 60$ , respectively). Mated males in poorer wing condition had larger testis weights. This trend is consistent with the findings of Herman et al. (1981) who found that male testis wet weight increases slightly, but steadily as males age. However, this finding is inconsistent with my finding that mated males were more likely to be in poorer condition, yet they did not differ in relative testes wet weight from non-mated males.

An unanticipated trend that became obvious during the dissections was that many mated males had depleted abdominal cavities (abdominal contents were dry and did not fill the cavity), and/or fragile and depleted (shriveled) reproductive tissues. I noted these males during the dissection. Using my subjective designations, I found that depleted abdominal cavities and depleted reproductive tissues were associated in dissected males ( $X^2$  (with continuity correction) = 16.52,  $p < 0.0001$ ,  $n = 120$ ). Both were associated with mating activity ( $X^2 = 9.01$ ,  $p = 0.003$ ,  $n = 120$ ,  $X^2 = 23.09$ ,  $p < 0.001$ ,

$n = 120$ ), for depleted abdomen and depleted reproductive tracts, respectively. Twenty-seven percent of the mated males had depleted abdominal cavities, compared to 3% of the non-mated males. Even more dramatic was the finding that 35% of mated males had apparently aged reproductive tracts, compared to none of the males in the general population.

Table 3-3 compares male wet weight, size, condition, relative testis wet weight, and relative tubular gland wet weight between males with either depleted abdomens or depleted abdomens versus all other males, regardless of their mating status. Depleted males were significantly lighter in wet weight, smaller in size, in worse condition, and had smaller relative tubular gland wet weights compared to all other males. Only relative testis wet weight did not differ between these groups.

Lipid weight, expressed as percent male dry weight, did not significantly differ between mated and non-mated males ( $Z = 1.61$ ,  $p = 0.11$ ,  $n = 28$ ). However, in support of an association between low lipids and male mating activity, male size was positively correlated with lipid weight ( $r_s = 0.40$ ,  $Z = 2.06$ ,  $p = 0.04$ ,  $n = 28$ ), while small size was associated with mating (this study, Chapter 2).

### Hypothesis 2: Small Male Takedown Advantage in Aerial Pursuit

A total of 27 trials had a winner in both the large and the small cage. Of these, small males were the first to takedown a female in the large flight cage 11% of the time, while large males were first in 89% of the trials. This trend is the opposite that expected if small



males have an aerodynamic advantage in pursuing females. Furthermore, this large male bias disappeared when the same 2 males were given the opportunity to copulate with a single female in the absence of the takedown component of precopulatory interactions. In small cages, small males were the first to successfully couple with females 56% of the time. The fact that small males were as likely as large males to successfully mate with a female in the presence of one other potential competitor, suggests that small males are equally vigorous and motivated to mate as large males. Undetected differences in vigor could differentially impact the flight component of the mating, but my observations suggest that this was not the case.

Because I tested the same pair of males in both the small and large cage, the critical test is to compare the combinations of winners in pairs flight versus no-flight cage treatments using a chi-square test with cage size as a repeated measure. Cage size, or opportunity for takedown, clearly influenced the outcome of male-female pairing ( $X^2$  (repeated measures) = 11.37,  $df = 1$ ,  $p = 0.0007$ ,  $n = 27$ ). The opportunity for takedown influenced which of the small-large male pairs was the first to mate, but in the direction opposite that predicted. Large males were at an advantage in takedown of free flying females, but there was no size related advantage in pairing with females when takedown was eliminated by the confinement of a small cage.

The mean wingloading (wet weight / (forewing length)<sup>2</sup>) of successful and unsuccessful males in the 27 paired male aerial takedown trials was  $0.220 \pm 0.004$ ,  $0.205 \pm 0.003$  SE, respectively.

Using the Mann-Whitney normal approximation Z test statistic, these were significantly different ( $Z = 2.82$ ,  $p = 0.005$ ,  $n = 54$ ). For comparison, the wingloading in Mexico overwintering mated and general population males collected during the mass mating period was  $0.165 \pm 0.0009$  SE,  $n = 389$  and  $0.163 \pm 0.0009$ ,  $n = 433$ , respectively. These did not significantly differ ( $Z = 0.73$ ,  $p = 0.46$ ). Males with higher wet weight:wing size ratios may be at an advantage in aerial pursuit, but this apparently cannot explain the small male advantage observed in Mexico.

### Discussion

#### Hypothesis 1: Large, Good Condition Males Do Not Attempt to Mate 1985 Male activity versus phenotype comparisons

Male phenotype did not differ between active and inactive samples collected during the mass mating event in Mexico. Large, good condition males were available to mate in all active category samples, including flying, drinking and nectaring males. Phenotypic differences in the propensity to take flight or to become active does not account for the small male mating advantage observed in 1985. Mating males are a distinct sub-set of active males.

#### 1988 Male reproductive tract development versus mating status

Differences between mating and non-mating male phenotypes were consistent with my findings from 1985 (Chapter 2). Overall,

mating males were smaller and older than non-mating males. Males that mated early in the mating period were smaller, lighter in wet weight and in worse condition than males in the general overwintering population. However, unlike in 1985, late in the mating period, mated males differed only in being in worse condition. This change through time occurred because the general population came to more closely resemble the mating population in phenotype.

In post-diapause, but not diapause male monarchs, tubular gland tissues increase in wet weight in response to changes in daylength and temperature. This increase is controlled by rising titers of juvenile hormone (Herman 1975, Herman et al. 1981). Testis wet weight is not responsive to juvenile hormone, but does increase slightly and then steadily decreases in summer breeding males (Herman et al. 1981). Therefore, if phenotypic differences in male reproductive state accounts for the observed small male advantage, mated males would be predicted to have higher average tubular gland wet weights, while they should not differ from non-mated males in testis wet weight. Instead, I found the opposite trend. Mated males had lower weight tubular glands than non-mated males, while there was also no difference in testis wet weight between mated and non-mated males. The use of relative weights suggests that these differences were not due to the smaller size of mating males.

Tubular glands and testis wet weights were comparable to those found by Herman et al. (1989) for Mexico late overwintering males. Both tubular gland and testis wet weight increased through

time in the general population, consistent with the expectation that more and more males break diapause through time. However, the tubular gland wet weight of mated males did not change through time and was lower than that of the males in the general population.

Because mated males are small and worn, I originally expected higher tubular gland wet weights in smaller and in poorer condition males. In contrast to my expectation, I found no association between male size and relative tubular gland wet weight in either mated or non-mated males. However, in mated males there appeared to be 2 distinct trends in body size versus relative tubular gland wet weights. Relative tubular gland weight was consistently very low small mated males, while larger males showed higher and more variable weights, similar to those found in the general overwintering population (see Figure 3-6). However, even in relatively large mated males there was a much higher percentage of males with very low relative tubular gland weights as compared to the general population. The overall distribution of relative tubular gland weights in mated males also showed a highly skewed distribution toward very low weights. In contrast, the distribution of mated male relative testis wet weights was normally distributed and did not differ from non-mated males (Figure 3-5).

These results suggest that 2 independent factors may together define the mating population. First, males in a non-diapause state, as indicated by low body weight, fragile and depleted reproductive tracts and associated with old age, account for a large portion of mating males. Second, males that break diapause, as indicated by increased tubular gland weights, also mate. In the mating

population, this second group of males are more likely to be large, and in good condition.

Poorer wing condition was associated with larger relative tubular gland wet weights in the general population, suggesting that relatively old males cease diapause before younger males. However, this trend did not occur in mated males. Instead, low relative tubular gland weights dominated in all wing condition categories. These results suggest that, although reproductive tract development may be associated with aging, or *vice versa*, this trend does not explain the association between poor wing condition and mating status.

Herman et al. (1981) found that male testis wet weight increases slightly, but steadily as males age. In this study, small male size was generally associated with heavier relative testis wet weights. Furthermore, poor male condition was associated with heavier relative testis wet weights in the non-mated male population. In light the findings of Herman et al. (1981), these trends suggest that small and poor condition males have old rather than underdeveloped reproductive tracts.

Old age and low lipid reserves were common in mated males, and very uncommon in non-mated males. Males with depleted abdominal cavities and/or reproductive tract tissues were lighter in wet weight, smaller in size, in worse condition, and had smaller relative tubular gland wet weights compared to all other males. Relative testis wet weight did not differ between these groups. The association between age-related phenotypic characteristics and fragile, low weight reproductive tissues suggest that these

apparently old males may not have undergone a reproductive diapause during the overwintering period. Their low weight tissues reflect spent rather than undeveloped tissues. This apparently accounts for the absence of an association between mating and tubular gland weight.

Male lipid weight, expressed as percent male dry weight, did not differ between mated and non-mated males; perhaps due to the small sample sizes ( $n = 14$  and  $14$ ; for mating and non-mating males respectively). However, small male size and poor condition were associated with low lipid levels, and both small male size and poor condition were associated with mating activity (this study, Chapter 2). Together these findings support an association between size, age, low lipid reserves, and mating activity. Alonso-M. (1996) also found that small, poor condition males were likely to have low lipid reserves.

#### Hypothesis 2: Small Male Takedown Advantage in Aerial Pursuit

A small male advantage in aerial pursuit was not supported under semi-natural conditions. Small males were much less likely to takedown females in aerial pursuit than large males. The large male advantage was evidently not a result of differences in mating motivation between the males or to female choice, since small males were equally likely to mate with a female in the absence of aerial pursuit. Both pre-tests and observations during the experiment suggest that differences in vigor did not account for the observed large male advantage.

Males successful in aerial takedown had higher wingloads (wet weight/(forewing length)<sup>2</sup>) than unsuccessful males. However, the mean wingloading of unsuccessful males was still higher than both mated and non-mated Mexico overwintering males. Furthermore, mated and non-mated overwintering males did not differ in wingloading. Higher wingloading may be advantageous in aerial pursuit. However, theoretically, larger weight loads should be disadvantageous in rapid flight maneuvering (references in Wickman 1992). Regardless, such an advantage cannot explain the small male advantage observed in Mexico. It is possible that an experimentally induced advantage related to a higher wing loading obscures a small male advantage unrelated to wingloading. However, the results of the reproductive tract dissections suggest that differences in reproductive state is a more parsimonious explanation for the small male advantage observed in Mexico.

There are 2 possible confounding problems with this experiment. First, it was necessary to use summer generation monarchs to test for differences in flight ability in overwintering monarchs in order to control for age and condition influences in flight ability and to observe aerial pursuit from initiation through capture. It is possible that there is a difference in size versus takedown ability between the populations. However, my objective was to simply test whether small size, *per se*, leads to a superior ability to track females in flight, all else being equal. The most critical control in this test is that all else be equal between each small-large male pair. Furthermore, I see no reason to expect differences in the relation between size and flight ability between overwintering and

summer generation monarchs. Differences between overwintering and summer generation monarchs, including age, condition, lipid reserves, and diapause state, both differ and likely influence likelihood of male takedown (see hypothesis 1), but these were specifically controlled in this experiment.

The second problem is more potentially troublesome. I tested male size versus flight ability under summer conditions with temperatures ranging between 26 and 33 °C, while aerial pursuit at overwintering sites occurs under much lower ambient temperatures. If small males have a relative advantage only under low temperatures, or large males have a relative advantage only under high temperatures, my findings would not reflect size-related flight ability under field conditions.

I did not monitor the temperature during the mating event in Mexico, but mating activity and flight in general were obviously constrained by temperature. Monarch flight threshold ranges from 11.7 °C to 16.0 °C ambient temperature (Masters et al. 1988, Alonso-M. et al. 1993). Maximum ambient temperatures in 1987 and 1988 ranged between 12 °C and 19 °C during the mass mating event.

Generally large butterflies (this usually refers to interspecific size variation) heat and cool more slowly and also lose heat more slowly (Douglas 1978, in Masters 1993). With regard to monarchs flying at relatively low temperatures, small males may be at an advantage in reaching flight threshold, but large males should better be able to maintain flight (references in Masters 1993). Mass mating occurs only when a large proportion of the overwintering colony is in



flight. The fact that monarchs collected in flight during the mass mating event were similar in size to those found clustered, but differed from those found as mated pairs, suggests that size-related capacity to fly at relatively low ambient temperatures cannot account for the small male mating advantage. However, small males may still be at an advantage in takedown ability under low temperatures.

Both a small and a large male advantage in mating at low temperatures has been reported in the insect literature. For example, in a gregarious nesting bee (*Colletes cunicularius* L. (Colletidae)), larger males are more likely to be found in mated pairs during periods of low ambient temperatures (Larsson and Tengo 1989). The authors, along with other published literature cited by them, suggest that relatively smaller insects require higher ambient temperatures to attain flight threshold. However, Larsson and Kustvall (1990) found that small male cerambycid beetles (*Stenurella melanura* L.) were more likely to mate during rising temperatures, while large males were more likely to mate at higher temperatures. They speculated that this difference was due to an inverse size-related ability to reach flight threshold by basking. However, they found no relationship between temperature and size in non-mated males or females collected from flowers.

My observations during the flight experiment, including an apparent increase in motivation and persistence by relatively older males, suggest that size advantage, *per se*, does not account for the small male advantage observed in Mexico. These results, taken together with the results of the male reproductive tract dissections,

strongly suggest that age and size related differences in reproductive state, rather than flight ability, account for the bias toward small males in mated pairs.

### General Discussion

Although large, good condition males left clusters and were collected in actively flying samples during the mass mating period, they were underrepresented in mated pairs in Mexico. The observed small, worn male advantage was not due to a small male flight advantage in aerial pursuit. Furthermore, I found no evidence of female choice in this study, or in Chapter 2, that could account for the observed small male advantage.

Phenotypic differences in reproductive state at least partially account for the small, worn male mating advantage observed in Mexico overwintering males. However, unexpectedly, male mating activity was associated with old rather than maturing reproductive tissues. Low reproductive gland weights, fragile tissues, depleted abdomens, and poor wing condition support an age-related effect on male propensity to mate in Mexico.

Herman and coworkers (for reviews see Herman 1985, Herman 1993), studying California overwintering monarchs, have shown that increases in juvenile hormone level, associated with increasing temperature and day length, results in reproductive tract development and rapid senescence in post-diapause, but not in diapausing, overwintering monarchs. However, the conclusion that rising juvenile hormone levels result in overwintering mating

behavior is based on data from males collected from the general population rather than from mating males. My results suggest that it is important to consider intraspecific variation in reproductive tissue development, and to specifically correlate such variation with male activity if we are to understand the connection between reproductive physiology and male mating behavior. Alternatively, there could be a difference in the reproductive physiology between eastern and western U.S. monarch populations.

Age and size are better predictors of mating activity in Mexico overwintering males than reproductive tract development. This is not to say that reproductive state of overwintering males is unrelated to mating behavior, only that the relation may not be straightforward and may not mirror summer breeders. Also, low reproductive tissue weights were associated with both diapause and possibly old non-diapausing, reproductively active males. Indicators other than tissue weight are therefore necessary to differentiate reproductive state.

#### The ontogeny of male mating tactics

Size and age were negatively correlated in both mated and non-mated males (Chapter 2). This association occurs very early on in the overwintering period (Alonso-M. 1996), suggesting that old males are more likely to be small. Alternatively, small males may break diapause sooner and thereafter age rapidly (see Herman 1985). However, using reproductive gland weights and condition as indicators, this alternative was not supported.

I suggest that old males with low lipid reserves and fragile low weight tubular glands represent a sub-set of monarchs that overwinter without entering reproductive diapause. Because summer monarchs occur as overlapping generations (Cockrell et al. 1993) and are distributed over such a large geographic area, late summer eclosing monarchs experience small to large differences in daylength and temperature. I hypothesize that variable environmental effects on body size and hormonal levels account for the association of size and condition and is an important source of phenotypic differences in male mating behavior at Mexico overwintering sites. The relative proportion of non-diapausing individuals will vary with environmental conditions at emergence sites (Lessman and Herman 1983, James 1993), during the autumn migration (Barker and Herman 1976), and, especially in the more mild overwintering areas in California and Australia, at the overwintering site (Malcolm 1987, James 1993, see Chapter 2).

Females mate before leaving summer breeding grounds (Lessman and Herman 1983), during the autumn migration (Williams 1942, pers. obs.), and early on in the overwintering period in Mexico and in California (Downes, in Williams et al. 1942, Hill et al. 1976, Tuskes and Brower 1978, Brower et al. 1977, Leong et al. 1995, Calvert pers. comm., Alonso-M. pers. comm.). Although these females may be in reproductive diapause, since males can potentially enforce copulations, I assume that the males that mate with these females are not in reproductive diapause. Since it is unlikely that males can reinstate diapause after becoming reproductive (Herman 1985, James 1993), the occurrence of mated females before the mass

mating period suggests that a portion of males overwinter in a non-diapause state.

Wing length is inversely related to the temperature under which monarchs develop (Arango-V. 1996). As temperatures cool in late autumn, eclosing monarchs are expected to be larger, with lower juvenile hormone levels, while monarchs that eclose earlier or those that occur in more southerly latitudes are more likely to be smaller and more likely to have developed reproductive tissues.

Herman (1988) presents supportive evidence of an environmentally induced size trend in field collected monarchs. As predicted from the laboratory findings of Arango-V. (1996), he found a dramatic increase in wing length and wet weight in both male and female monarchs that eclose very late in the summer in Minnesota relative to those that were collected mid-summer. Wing size and wet weight peak in late-August, when migration begins. Interestingly, by mid-September mean wing length again decreased. My hypothesis of age-related reproductive behaviors suggests that these small monarchs occurring late in the migration period should be older and in worse condition. Unfortunately, Herman did not report wing condition. Also, because he used hindwing length rather than forewing length, I cannot compare his data to mine.

A tendency for small, perhaps older, males to leave late summer breeding grounds after large males is suggested by the work of Beall (1946). He showed that as the sex ratio became more female biased in Ontario, Canada in mid-September, the average male size decreased such that males were smaller than females. Males were larger than females before the seasonal change in sex ratio. Small,

worn, reproductive males may postpone autumn migration and mate with females remaining at summer breeding sites. Unfortunately, he did not indicate wing condition. However, Brown and Chippendale (1974) found that autumn field collected Missouri adults with longer wing lengths and heavier wet weights had significantly more lipid reserves than smaller monarchs. High lipid reserves are associated with diapause state and low reserves with reproductive activity (Dallmann and Herman 1978).

Further evidence of the occurrence of a mixture of reproductive states in monarchs during autumn migration comes from Lessman and Herman (1983). They showed that juvenile hormone level, indicative of monarch reproductive state, is highly variable from late August through October in both males and females. They also found a decline in mating associated with decreased juvenile hormone levels and the initiation of the southward migration. This was followed by a second peak in mating and associated rise in juvenile hormone level in October; by which time monarchs are already arriving to Mexican overwintering sites. It would be interesting to know whether the variation in juvenile hormone level and mating activity reflect monarch size and condition.

Beall (1946, 1948) offers evidence that small, older males may be especially likely to succumb to the rigors of the southward autumn migration. Monarchs found dead on the Canadian shore of Lake Erie on 13 September 1943 were significantly smaller, lower in lipid weight, and lower in lean weight than monarchs collected from clusters beside the lake shore.

In temperate insect populations, in which some individuals in the penultimate generation develop directly into reproductive adults while others enter diapause, males are expected to enter diapause before females. Using Evolutionary Stable Strategy reasoning, such males are expected to gain reproductively by emerging in the spring before females (Wiklund et al. 1992). In an hypothesis put forth to explain male-biased sex ratios at California overwintering sites and following Wiklund et al. (1992), Nylin et al. (1995) predicted that male monarchs should start and end diapause before females. Although there is no evidence that males that enter diapause early also end early, diapause lasts longer in females than males (references in Herman 1985, 1993). If, as a result of differences in propensity to enter diapause, females remain at summer breeding sites longer, they could provide an alternative reproductive option for small males. Males could also mate with females that emerge in diapause since they can potentially force copulations on females, perhaps inducing them to oviposit (Herman and Barker 1977). In *Eurema hecabe*, a seasonally dimorphic butterfly, female autumn morphs mate primarily with male summer morphs (Kato 1989).

Summer mating males would be expected to experience much lower costs in obtaining mates by mating before migration, but these males would be expected to have lowered reproductive payoffs compared to males that migrate. This is because the ensuing deterioration of environmental conditions will either force females to migrate before ovipositing or will cause greatly reduced offspring survival. However, summer breeding males may salvage reproductive fitness if they can still successfully migrate and

overwinter, and mate again before, or instead of, remigrating. Since these males are not in reproductive diapause they are expected to be reproductively active early on in the overwintering mating period. This hypothesis is consistent with my findings.

In contrast to summer breeders, late summer generation males that go directly into diapause will forfeit summer reproduction and early overwintering breeding. They are expected to experience higher migration and mate location costs, but their investments should be much less risky due to last male advantage in sperm precedence. At overwintering sites, diapausing males should be selected to mate just before females remigrate due to last male advantage in sperm precedence. However, if they wait too long, they risk never mating due to the high costs of remigrating and locating females at spring oviposition sites. Assuming that males can mate multiply but that females rarely do, diapausing males should also break diapause before females to maximize mating opportunities while mate location costs are low. This hypothesized scenario could account for the exponentially increasing mass mating activity that I observed at a Mexico overwintering sites (Chapter 5). It is also consistent with observed changes toward higher proportions of females in late summer breeders (Beall 1946, Eanes and Koehn 1978), and late Mexico overwintering monarchs (Chapter 2, Chapter 5).

Extremely male-biased late overwintering sex ratios in California do not follow this pattern. However, differences in late summer conditions and the much shorter distance to overwintering sites could tip the scale toward females investing more in summer



breeding. This would be expected if summer breeding was less likely to negate successful autumn migration in short versus long migrations. The occurrence of such females should favor males that remain longer before or instead of migrating to overwintering sites.

Once sexually active, monarchs apparently do not reinstate reproductive diapause (Herman 1985, James 1993). Associated with the non-diapause state is a decreased capacity to store lipids and an accelerated senescence (Dallmann and Herman 1978, Herman 1985, Herman 1993), presumably for both eastern and western U.S. monarchs. These may in turn lead to an earlier cessation of overwintering diapause (Herman et al. 1989). Assuming relatively more males invest in summer reproduction, do not undergo diapause, and age more rapidly, California overwintering males would be expected to invest more in overwintering reproduction. This is because old, poor condition males have less future reproduction to lose, and can not afford the costs of remigration. This could account for the male-biased sex ratio in California late overwintering populations. This hypothesis is amenable to field testing.

Although monarchs collected during colony formation in the autumn in both Mexico and California generally had high lipid contents, some arrive at these sites with low levels (Tuskes and Brower 1978, Alonso-M. 1996). Alonso-M. (1996) found that flower visiting monarchs collected early in the overwintering period were smaller and had less than half the level of lipids found in clustered monarchs. Furthermore, nectaring monarch lipid levels were similar to those found in non-migratory summer generation monarchs,

suggesting that nectaring monarchs arrive in Mexico with low lipid levels.

### Do non-diapausing monarchs remigrate?

Non-diapause monarchs are expected to be less likely to survive the overwintering period. Calvert and Lawton (1993) and Alonso-M. (1996) give evidence that small, poor condition monarchs are more likely to die during the Mexico overwintering period. James (1981) also found differential mortality of old monarchs in a non-diapausing Australian overwintering population. However, presuming these non-diapause males make it to overwintering sites and survive until the mass mating period, they would be expected to be both physiologically ready and evolutionarily selected to mate, i.e. they would have developed reproductive glands, rapid senescence, low lipid reserves, and poor wing conditions, making post-remigration mating risky and costly.

In order to test whether small males are less likely to remigrate, I compared wing length of successful remigrators with late overwintering Mexico monarchs collected both from the general population and as mated pairs in 1985. I used cardiac glycoside fingerprint patterns produced by Malcolm et al. (1993) to differentiate overwintering from early spring generation monarchs collected in the southeastern United States. Adult monarch cardenolide fingerprints reflect the milkweed species upon which larvae fed. Because 92% of Mexico overwintering monarchs show *Asclepias syriaca* fingerprint patterns, this pattern can be used to

differentiate spring remigrants from the first spring generation monarchs which feed on a variety of other milkweeds.

Based on data in (Malcolm et al. 1993, and their unpublished raw data), I selected 58 spring migrants collected before 21 April 1985 with the *A. syriaca* pattern. The sex ratio of these successful remigrators was 55% males, indicating that males do remigrate with females. This is in contrast with mark-recapture data from remigrating California monarchs (Nagano et al. 1993). There, females were recovered more often than expected from the overwintering sex ratio.

I compared these early remigrants with general population and mated males collected during the mating period (11 Feb - 25 Mar) from the Mexico overwintering site in 1985. The distribution of male wing length among these collection categories is shown in Figure 3-7. Using Mann-Whitney normal approximation Z tests, I found no difference in wing length between successful remigrants and those in the general overwintering population ( $Z = 1.08$ ,  $p = 0.28$ ). However, mated males were significantly smaller than successful remigrants ( $Z = 2.19$ ,  $p = 0.03$ ). Although small, worn males are found in the overwintering period as mated pairs, the data indicate that they do not cluster with the general overwintering population, and many of these, especially the relatively small ones, do not successfully remigrate (see Figure 3-7).

Alonso-M. (1996) also found no difference in wing length between successful southern U.S. remigrants and late Mexico overwintering monarchs, using combined sexes and years (1985 - 1986). However, remigrant monarchs had longer wings than flower-

visiting late overwintering monarchs, consistent with my results of mated versus remigrant males. Alonso-M. also showed that flower visiting monarchs were nearly devoid of lipids at the end of the overwintering period; an indication that nectaring monarchs are unlikely to be able to remigrate. My field work did not show a size-related tendency for males to visit nectar sources in 1985 (see Table 2-2, Chapter 2; and Figure 3-4), but nectaring males did resemble mating males in having relatively low body weights and poor wing conditions. Furthermore, nectaring male (Alonso-M. 1996), and mated male (see Figure 3-7) size distributions were skewed toward small males not represented in the general overwintering population. Alonso-M. suggested that these small males may roost outside of the colony or very high in the trees unreachable by our sampling methods.

Together, this study and the findings of Alonso-M. suggest that 1) nectaring and mating males may be the same or a similar sub-set of the general overwintering population, 2) that small, poor condition monarchs arrive at the overwintering site reproductively active, and 3) that males which had never entered diapause are less able to survive the overwintering period and successfully remigrate.

Following the argument of Odendaal et al. (1985), I suggest that males with little or no opportunity to mate after the mass mating period should expend energy almost exclusively to mate, while forfeiting energy conservation behaviors such as clustering. Clustering could not only reduce opportunities for mating, but could result in the dying of monarchs with near zero lipid reserves if low temperatures and shade prevent clustered individuals from taking

flight. In fact, the unresolved question of why monarchs are generally so active at the end of the overwintering period, when they should be conserving energy for remigration (see Masters et al. 1988), may be related to the balance of mate acquisition versus remigration energy budgeting. This may also be true of females. However, the similar sex ratios in flying versus clustered monarchs together with the low proportion of mating in females (Chapter 2) argue against this possibility in Mexico.

### Conclusions

The costs and benefits of mating for males at the overwintering site are expected to reflect the interaction of environmentally induced phenotypic differences and environmentally dependent fitness effects of mating before, during or after the overwintering period. The latter includes the effects of the timing and frequency of female mating. Both the results of this study and the work by Herman et al. (1989) suggest that the timing of breaking diapause, age, condition, size, and their interaction are important determinants of which males mate in the overwintering population.

Overwintering females mate before, during and after the overwintering in Mexico (Chapter 5). The frequency of mating and the degree of multiple mating is relatively low in overwintering females compared to spring remigrants and summer breeders. Males that mate in Mexico therefore may sacrifice paternity assurance for reduced mate location costs and avoidance of remigration costs. Furthermore, they may have little future reproduction to lose and

therefore should invest more heavily in reproduction than young, larger males. However, unlike relatively young males that gain reproductively only after remigration, these males' reproductive success potentially also includes offspring sired in the late summer, during the autumn migration, and early on in the overwintering period. Understanding the proximate factors influencing monarch reproductive success and documentation of male and female mating patterns throughout their life history cycle and across years will be necessary to understand the demographic and possible evolutionary effects of phenotype-dependent overwintering mating behaviors (Dunbar 1982).

The interaction between the environment, phenotype, and differences in male and female reproductive strategies can be complex. For example, males takedown females and can potentially force copulations on unreceptive females. Male takedown ability and female ability to resist attempted copulations are expected to vary with their phenotype. Forced copulations may be against the reproductive interests of unreceptive females (Clutton-Brock and Parker 1995). Female receptivity, in turn, depends on environmental conditions such as temperature and daylength, phase of the life history cycle, and possibly also with mating history and age. The potential conflict between male and female reproductive interests (*sensu* Parker 1979) may therefore also vary through space and time (Clutton-Brock and Parker 1995). Likewise, which sex controls the outcome of attempted copulations may also vary with female receptivity and environmental conditions (Ridley 1990).

Environmentally induced variation in the interaction between male takedown, female receptivity, and which sex controls the outcome of mating attempts may contribute to the differences in the frequency of female mating and the degree of polyandry among different monarch overwintering populations, and during different phases of the life history cycle. The monarch butterfly offers a prime opportunity to study the influence of environmentally induced individual differences in mating behavior, since Mexico, California, and Australia overwintering monarchs experience different environmental conditions before, during and after they reach the overwintering sites.

Table 3-1. Male adult weight wet, forewing length, and wingloading (wet weight/forewing length<sup>2</sup>) in starved versus non-starved larvae (\* = p value < 0.0001).

Character	Starved			Non-Starved			df	Z	p
	n	Mean	SE	n	Mean	SE			
Wet Weight (mg)	93	498	9.5	110	624	5.8	201	9.3	*
Forewing Length (mm)	93	50	0.3	110	55	0.1	201	8.9	*
Wingloading <sup>2</sup> (mg/(mm) <sup>2</sup> )	93	20	0.2	110	22.0	0.2	201	7.8	*



Table 3-2. General population versus mated male wet wt., wing length (mm), wing condition (no units), relative testis wt. (testis wet wt./male wet wt.)\*100), and relative tubular gland wt. ((tubular gland wet wt./male wet wt.)\*100) in early and late Mexico overwintering mating season samples: 1988. All wet weights in mg.

	General Population			Mated Pairs			Z	p
	n	Mean	SE	n	Mean	SE		
Early								
Wet Wt.	20	543	15.1	21	502	1.56	2.02	0.04
Wing Length	20	53.0	0.3	21	51.2	0.5	4.67	0.0001
Wing Condition	20	2.0	0.2	21	3.6	0.2	4.44	0.0001
Rel. Testis Wt.	20	0.63	0.03	21	0.62	0.04	0.08	0.94
Rel. TG Wt.	20	1.70	0.14	21	2.44	0.42	0.31	0.75
Late								
Wet Wt.	19	476	13.9	20	454	236	0.34	0.74
Wing Length	19	51.7	0.5	20	51.5	0.9	0.11	0.91
Wing Condition	19	2.7	0.2	20	3.5	0.2	2.33	0.02
Rel. Testis Wt.	19	0.83	0.5	20	0.67	0.4	2.44	0.01
Rel. TG Wt.	19	3.64	0.36	20	1.57	0.17	4.47	0.0001

Table 3-3. Comparisons of males with depleted abdomens or reproductive tracts (n = 30) versus all other males, irrespective of mating status (n = 90), wet wt. (mg), wing length (mm), wing condition (no units), relative testis wt. ((testis wet wt./male wet wt.)\*100), and relative tubular gland wt. ((tubular gland wet wt./male wet wt.)\*100) in Mexico overwintering mating season samples: 1988.

	Depleted Males		All Other Males		Z	p
	Mean	SE	Mean	SE		
Wet Wt.	443	18.7	514	7.63	3.53	0.0004
Wing Length	50.4	0.7	52.3	0.22	2.82	0.005
Wing Condition	3.3	0.2	2.8	0.1	2.65	0.008
Rel. Testes Wt.	0.64	0.04	0.7	0.02	1.55	0.12
Rel. TG Wt.	1.97	0.2	2.68	0.18	2.08	0.04

## Male Gonad Development

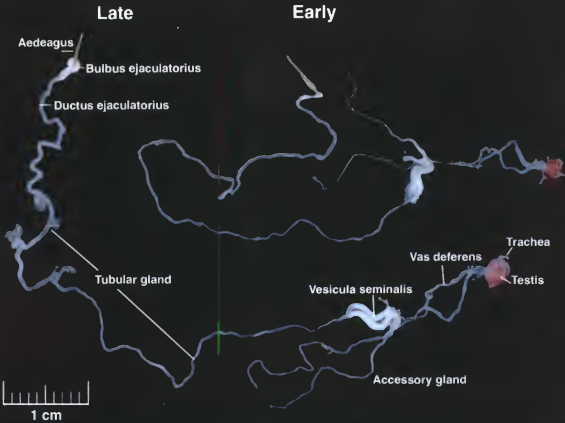


Figure 3-1. Photograph of representative reproductive tract development in males collected in the early (25 - 26 February) versus late (22 - 23 March) mating period, in Mexico overwintering monarchs, 1988. Reproductive tract nomenclature is after Urquhart (1960). Reflecting the terminology of Herman (1975b), I refer to the entire length of the common duct from the vesicula seminalis to the aedeagus as the tubular gland. This length consists predominantly of the tubular gland, but also includes the ductus ejaculatorius and bulbus ejaculatorius as described by Urquhart (1960, p. 246). The relative tubular gland wet weight and relative testis wet weight were used as measures of male reproductive state (see text). The testis was cut from the vas deferens, and the tubular gland was cut at one end from the junction of the vesicula seminalis and at the other end from the aedeagus. The ductus ejaculatorius and bulbus ejaculatorius are not externally distinct from the tubular gland and also increase in weight in response to juvenile hormone. These were included in the tubular gland weight in order to maintain sampling consistency. The original photographic slide image was altered on Adobe Photoshop version 3.0.1 for Macintosh to improve clarity.

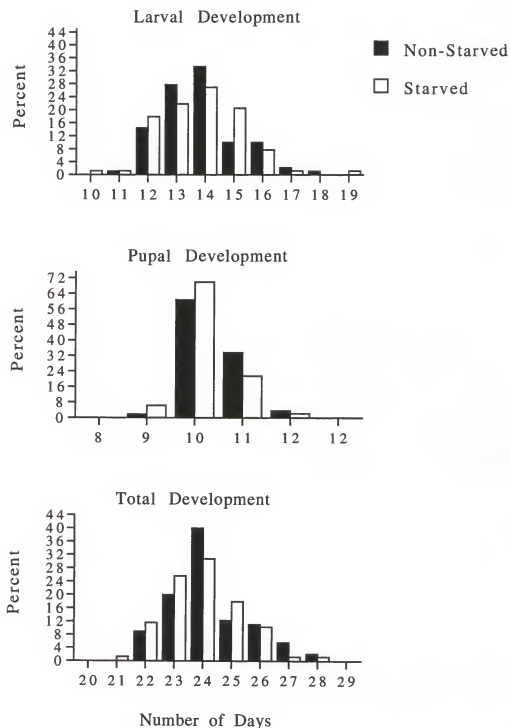


Figure 3-2. Comparison of developmental times (days) of starved versus non-starved larvae: Larval = egg hatch to pupation, Pupal = pupation to eclosion, Total = egg hatch to eclosion (n = 90, 110, 90, respectively).

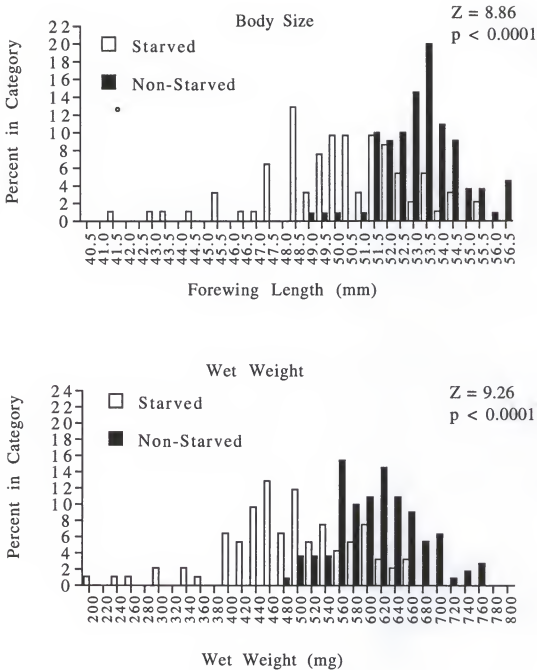


Figure 3-3. Adult male wing length and wet weight distribution of starved versus non-starved larvae ( $n = 93$  and  $110$ , for starved and non-starved males, respectively).

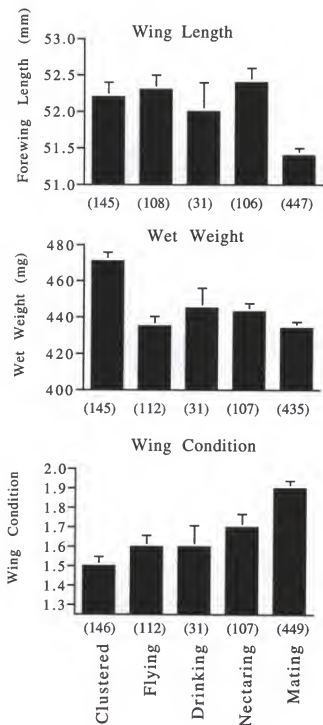


Figure 3-4. Overwintering male activity *versus* phenotypic characteristics during the mating period (10 February - 23 March 1985). Larger value wing condition = worse condition (see text). Error bars represent standard error.

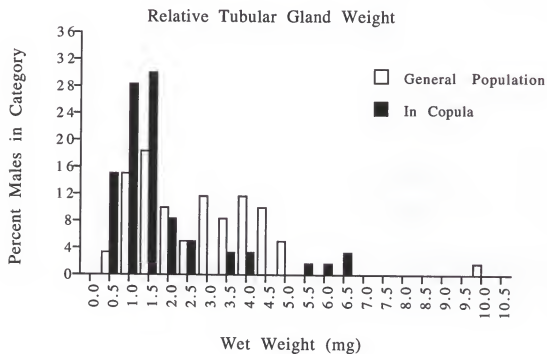
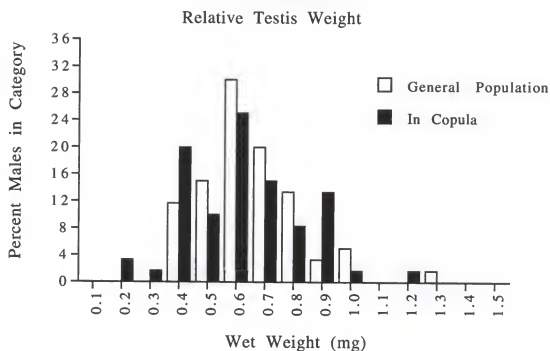


Figure 3-5. Reproductive tract development in mated versus general population males: 1988 (n = 60 for all categories).

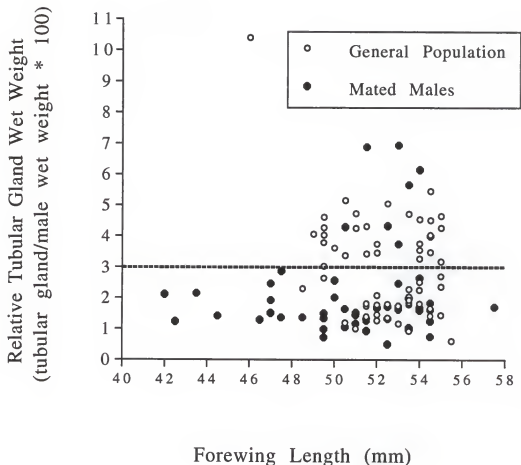


Figure 3-6. Male size versus relative tubular gland wet weight in overwintering general population and mated males, Mexico, 1988. (Spearman rank correlation tests not significant at  $p = 0.05$  for either general population or mated males, see text). For comparison note that 48% of general population males have relative tubular gland wet weights above 3.0 (above the dashed line) compared to only 13% of mated males.



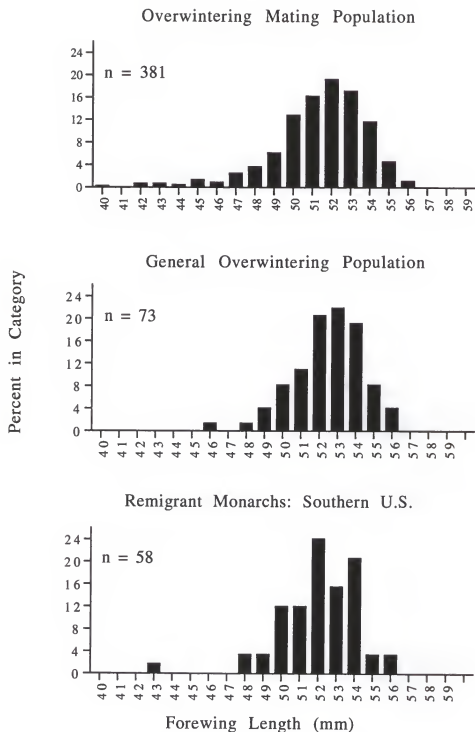


Figure 3-7. Comparison of male size among Mexico overwintering mating males, general overwintering males, and SE United States remigrant males: 1985. General population and mating males were collected from 5 March-25 March. Remigrating males were collected in April.

CHAPTER 4  
THE USE OF BURSA COPULATRIX DISSECTION AND  
ABDOMINAL PALPATION TO ASSESS FEMALE MONARCH BUTTERFLY  
MATING STATUS

Introduction

The monarch butterfly mates under distinctly different ecological conditions during the summer breeding and the overwintering mating period. Summer breeding is characterized by overlapping generations in dispersed populations associated with the larval food plant distribution. In contrast, the overwintering mass mating event follows a long-distance southward migration and reproductive diapause and occurs under extremely dense aggregations just before remigration (Brower 1985, Wells and Wells 1992). Females leaving overwintering areas are almost exclusively responsible for the initial re-population of summer breeding sites in the U.S. and Canada (Malcolm et al. 1993, Brower 1995).

Late summer generation females are reported to be in reproductive diapause and unmated (see Herman 1985). However, female mating has been observed before leaving summer breeding grounds (Lessman and Herman 1983), during the autumn migration (Williams 1942, pers. obs.), and early in the overwintering period in Mexico and in California (Downes, in Williams et al. 1942, Hill et al. 1976, Tuskes and Brower 1978, Brower et al. 1977, Leong et al. 1995, Calvert pers. comm., Alonso-M. pers. comm.). Furthermore, females arriving at California overwintering sites are occasionally

mated (Downes, in Williams et al. 1942, Tuskes and Brower 1978, Leong et al. 1995), and non-virgin females predominate at Australian overwintering sites throughout the overwintering period (James 1984b, James 1993).

A sub-set of overwintering females mate or re-mate at the overwintering site. Mating may occur during the remigration, and mating is known to occur when females reach oviposition sites. Females incorporate spermatophore-derived nutrients into their somatic tissues and eggs (Boggs and Gilbert 1979, Wells et al. 1993), and polyandry is common (Pliske 1973, Hill et al. 1976, Suzuki and Zalucki 1986, Oberhauser 1989, pers. obs.). In laboratory studies there is strong, but incomplete, last male mating advantage in egg fertilization (Oberhauser pers. comm.).

Knowledge of when female monarchs receive spermatophores is therefore important in understanding 1) both male and female mating strategies, 2) remigration population demographics, and 3) selective pressures that shape and maintain the migration-diapause life history strategy. The primary objective of this paper was to develop methods to 1) determine the frequency of mating and the degree of multiple mating in Mexico overwintering females, and 2) to differentiate matings that occur during the spring mass mating period from those that occurred late in the previous summer, during the autumn migration, or early on in colony formation.

Specifically, I assessed the usefulness of bursa copulatrix dissection and female abdominal palpation to determine female mating status. Daily abdominal palpations of just-mated females were used to determine the rate of spermatophore degradation

through time. This assessment increased my ability to differentiate between female that mated during the mass mating event from those that mated before that time. I also evaluated 2 methods for determining female mating frequency: 1) the number of spermatophore necks, and 2) the number of spermatophore stems. I also developed a dissection protocol that allowed detection of degraded spermatophores.

### Methods

#### Bursa Copulatrix Dissections

I dissected the bursae of 669 females collected between 15 January and 25 March 1985. I randomly sub-sampled equal numbers of clustered, flying, nectaring, and drinking females from the daily samples that I had palpated. Collection dates, activity category, sample size and percentage of the combined weekly dissection samples collected from each activity category are given in Table 4-3.

Females were killed by cutting the head from the body and stored on ice at my camping site until dissected within 3 days under a 10-30X binocular dissecting microscope. The abdomen was cut from the thorax to facilitate manipulation under the microscope. The abdominal exoskeleton was split longitudinally by sliding dissecting scissors just under the exoskeleton and cutting from anterior to posterior. With the exoskeleton pinned open on a dry paraffin surface, the bursa copulatrix was removed from the abdomen by

cutting the ductus bursa at the exoskeleton wall. The wall of the bursa was then peeled open and the bursa contents exposed.

At the time of the dissection, I 1) sketched a picture of the contents of the bursa, 2) recorded the color, shape, size, and viscosity of the contents, 3) noted the presence or absence of spermatophore wall pieces and the number of chitinized spermatophore necks, and 4) weighed intact spermatophores to the nearest 1 mg of intact spermatophores, using a Sartorius Model 1205 MP balance. The spermatophore necks are composed of hardened chitin and I saw no evidence of their deterioration, even in females with no other remaining parts of old spermatophores. I used either the chitinized neck or torn spermatophore wall pieces to confirm the presence of a completely disintegrated spermatophore. I practiced these methods before beginning the actual samples dissections in order to increase my accuracy. I used detailed descriptions and drawings to categorize spermatophores as fresh, semi-fresh or old after I had dissected the entire sample. To obtain wet weights of intact spermatophores, I dissected the bursa without adding water.

After dissecting all females, I used my sketches of the bursae contents to classify spermatophores as fresh, semi-fresh or old. Fresh spermatophores had turgid walls with full contents and the skin entirely intact. Because each fresh spermatophore is a distinct entity, the number present in the bursa copulatrix could be determined with certainty. Old spermatophores were relatively small, deflated, and their walls were usually frayed or broken into pieces. Extremely degraded spermatophores were detected by the presence of spermatophore wall pieces or chitinized necks. Once

spermatophores were physically broken into many pieces, I was only able to estimate the number of matings by the number of spermatophore necks found. When the contents of the bursa were old and brittle the dry dissection method may have prevented reliable detection of chitinized spermatophore necks and so I may have underestimated the mating frequency in such females.

However, I later determined that this caused minimal error (see below). Semi-fresh spermatophores were intermediate between fresh and old spermatophores in size, turgidity and deterioration of the walls. Figure 4-1 shows representative examples of fresh, semi-fresh and old spermatophores, and a chitinized spermatophore neck.

These spermatophore classifications were used to differentiate matings assumed to have occurred during the spring mass mating period (fresh and semi-fresh) from those assumed to have occurred previously during late summer, during autumn migration, or during overwintering colony formation. Dissections of females that were collected *in copula* (unpubl. data), changes in spermatophore mass as detected by palpation (this Chapter), and dissections of spring remigrants and summer breeders support these designations (see also Oberhauser 1992, Svård and Wiklund 1988a).

### Female Abdominal Palpations

Males transfer a large spermatophore to the female bursa during mating so that the mating status of live females can be assessed by palpation. This involves gently applying pressure between the thumb and index finger while feeling along the abdomen from

anterior to posterior for a hardened mass in the bursa copulatrix (Tuskes and Brower 1978). Mating status was categorized as virgin, mated, or freshly mated based on the relative size and hardness of the spermatophore inside the bursa.

I palpated a total of 3205 females collected between 1 February and 25 March 1985. In 1986, 1987, and 1988 I palpated a total of 2690 females between mid-February and late March. Table 4-2 gives sample sizes among activity categories for 1985-1988.

## Results

### Designation of Old Matings versus Virgins using Bursa Copulatrix Dissections

Females without evidence of chitinized necks or deteriorated spermatophore contents were classified as virgins. However, a subset of these contained a yellowish, translucent, waxy material in the bursa similar to that found in spermatophores. All other females classified as virgins had empty or fluid-filled bursae. If the waxy material remains after spermatophores have completely deteriorated and the chitinized spermatophore necks are undetectable, then these females would be incorrectly classified as virgins. Because these ambiguous bursa classifications made up 33% of all dissections, their classification is important in describing the incidence of old spermatophores in overwintering females. If these females were classified incorrectly as virgins, the estimated proportion of females with old spermatophores is vastly underestimated.

To reduce this potential error, I modified my method to include dissecting the bursa under water and sonication of the bursa contents (described below). Because this method vastly improved my ability to detect the tiny spermatophore necks, I used this method to assess whether the waxy material represents spermatophore contents (old matings) or secretions inside the bursa of virgin females.

Using the improved methodology, I dissected 47 females collected on 17 and 24 January 1985 from tree clusters using the same dissection methods as described above with the following changes. First, before dissection I inspected the females under a 10-30X dissecting microscope for the presence of spermatophore stems protruding from the genital opening. The presence of spermatophore stems, which extend from the spermatophore neck in fresh spermatophores and remain in the ductus bursa, is a definite indication of mating. Second, I determined the mating status inspection of the bursa contents. I then placed the bursa on a watch glass and flooded it with distilled water. The contents were again inspected under the microscope for the presence of spermatophore necks and wall pieces. Third, I washed the bursa contents into a 9 ml glass tube and sonicated it in a Bransonic 12 sonicator for approximately 5 minutes. The sonicated contents were washed into a second watch glass and again inspected under the microscope. Materials were removed upon positive identification and water replaced with a pipette until the remaining contents were clear enough for positive identification. This greatly facilitated detection of spermatophore skins and necks within the bursa contents.



Of the 47 females dissected using the new methods, 33 females (70%) had no spermatophore stems, necks, scraps of spermatophore wall material, or obvious spermatophore contents without wall pieces. I consider these confirmed virgins. Twenty-three (70%) of these 33 virgins had bursae that were either empty or contained clear fluid, another 4 (12%) had only waxy material and 6 (18%) had a combination of waxy material and fluids. It therefore appears that the waxy material can be present in the absence of mating. This supports my assumption that the females in the 1985 dissections that contained only waxy material without chitinized necks were correctly classified as virgins.

Of the 47 females, I found chitinized necks in 10 bursae (i.e. 21% were mated), and 9 of these had obvious stems protruding from the genital opening. Three of these bursae contained no other materials other than a chitinized neck, 1 contained fluids, 4 had waxy material outside of the spermatophore walls, and 2 contained both fluid and waxy material. These data indicate that fluids and waxy material may be present in both virgins and mated females but that the presence of chitinized necks is necessary to verify old matings. Again, this supports my method requiring the presence of a spermatophore neck or spermatophore skin pieces to classify a female as definitely mated.

Four of the 47 dissected bursas (9%) contained spermatophore contents that were intact enough for positive identification, in which no spermatophore stems, necks or wall pieces were detected. These results suggest that either the stems and/or necks sometimes disintegrate completely, or the presence of necks and stems can be

missed with my dissection methods. In my history of dissections of over 2000 bursae I have never seen evidence of deterioration of the spermatophore neck. I therefore assume that 1) either the neck was present but overlooked in the dissection or 2) the spermatophore may have been secreted into the bursa without the formation of a chitinized neck. In either case, these results suggest that up to 9% of females classified as virgins based on the presence of waxy material without spermatophore necks or wall pieces had in fact mated. In agreement with this error estimate, in 225 bursa copulatrix dissections of spring remigrating monarchs collected in 1986, the percentage of females found with fewer spermatophore necks than stems protruding from the genital opening was 16 (7%).

Of the original 451 females classified as virgins, 221 (49%) had waxy material but no other evidence of mating while 230 (51%) were definitely virgins. If 9% of the 221 females containing only waxy material had in fact mated, then approximately 20 of all 451 females (4%) classified as virgins were classified incorrectly. Therefore, the overall percentage of virgins may have been slightly overestimated (451/669, or 67.4% versus 431/669, or 64.4%), and the percentage of females with old spermatophores may be slightly underestimated (155/669, or 23.2% versus 175/669, or 26.2%).

#### Palpation Accuracy and Spermatophore Degradation through Time

According to Oberhauser (1992), under summer conditions, just-transferred spermatophore contents shrink at a rate of approximately 3.3 mg/day for the first 7 days, after which their

weight stabilized at approximately 5 mg for at least 20 days. Because of this shrinkage, I needed to estimate how long recently mated females retained spermatophore masses large enough to be palpated as fresh matings. I palpated 22 females collected from *in copula* pairs in 1988. The following day, after the male and female separated, I placed the females in a mesh cage in the shade. I misted the cage daily with water but did not provide females with a sugar source. I palpated each female at 24 h intervals for 2 weeks and noted the date when my subjective assessment, based on size, and hardness of the perceived spermatophore, changed from fresh to old.

All 22 females were classified as freshly mated for 7 or more days, and 5 (22%) remained in the freshly mated category on day 14 when the experiment had to be terminated. The estimate of the number of days just-mated females remain in the freshly mated category is therefore very conservative. Excluding these 5 females, the median number of days that females remained in the freshly mated category was 11 and the mode was 10. However, actively flying, nectaring, or courted females may utilize spermatophores much more quickly.

Detection of fresh spermatophores for approximately 2 weeks after mating, together with an absence of freshly mated females (as determined by bursa copulatrix dissection) before mass mating was observed in the field, suggests that palpation can differentiate between matings that occur during the spring mass mating period from those that occurred during the prior summer or during the autumn migration.

Because palpation of just-mated females can change from fresh to old in as little as 7 days, and the mating period lasts approximately 4 weeks, abdominal palpation is expected to slightly underestimate the true percentage of mating during the mass mating period, especially toward its end. However, because mating frequency builds exponentially (see Chapter 5, Figure 5-3) and by far most mating occurs in the few days before colony breakup, the underestimate caused by spermatophore shrinkage is expected to be small.

#### Palpation versus Bursa Copulatrix Dissection

I assessed the accuracy of my 1985 abdominal palpations in distinguishing mated females (including fresh, semi-fresh and old spermatophores) from virgins before mass mating began in mid-February by comparing palpation with dissection mating status classifications of the same female. The 221 females that had ambiguous bursa copulatrix contents, and therefore could not be definitively classified as virgin or non-virgin, were excluded from this comparison. In matched samples, 77% ( $n = 262$ ) of abdominal palpation results were consistent with dissection results in detecting the presence of deteriorated spermatophores. I was more likely to classify a female with an old spermatophore as a virgin (29%,  $n = 116$ ) than to classify a virgin as mated (18%,  $n = 146$ ) ( $X^2$  with continuity correction = 4.21,  $p = 0.04$ ,  $df = 1$ ,  $n = 262$ ). Since these incorrect classifications will effectively cancel each other out and there is a much higher percentage of virgins than females with old

spermatophores (see Chapter 5), palpations in the field are expected to slightly underestimate the frequency of females with old spermatophores.

Because abdominal palpation is better able to detect large, hardened fresh spermatophores than small, deteriorated old spermatophores, I separately compared abdominal palpation with dissection results on matched samples of recently mated females (fresh + semi-fresh spermatophores only) during the mass mating period (females collected on or after 20 February). Not surprisingly, mating assessment by abdominal palpation was more accurate in detecting recently mated females than females with old spermatophores. Ninety-four percent of 33 freshly mated females were palpated correctly. These results suggest that abdominal palpation is a useful non-invasive tool for assessing mating status, especially of recently mated females.

## Discussion

### Bursa Copulatrix Dissections

In this study I assume that each mating results in a single spermatophore and that spermatophore necks remain intact (see Pease 1968, Ehrlich and Ehrlich 1978, Lederhouse et al. 1989, Chapter 3, for support of these assumptions). My dissection method provides a good separation of virgins from females with old spermatophores. Dissection methods are more accurate than palpation in determining the relative time of when females receive

multiple spermatophores, especially when old spermatophores are very degraded. However, even using dissection, some females with completely deteriorated spermatophores and no detectable stems or necks may be classified incorrectly as virgins. New dissection methods that include inspection for spermatophore stems and sonication for better detection of spermatophore necks should effectively alleviate this potential error.

Bursa copulatrix dissection provides a means to differentiate matings that occurred during the mass mating event from those that occur much earlier in the overwintering season, late in the summer, or during the autumn migration. Differentiation of matings that occur during these different times rely on spermatophore size and degree of degradation.

#### Abdominal Palpations

Abdominal palpation provides a quick and relatively accurate method for assessing female mating status and does not require killing the female. Abdominal palpation is more accurate in detecting fresh (94%) than old spermatophores (77%). The occurrence of both old and fresh spermatophores are likely underestimated due to degradation in the bursa copulatrix. Palpation accuracy will depend on the initial size and degradation rate of the spermatophore, and on female intermating interval (see also Oberhauser 1992).

The reduced accuracy of old spermatophore detection arises from the inability to 1) differentiate between recent matings and old

matings, and 2) between very old matings and virgins. Tuskes and Brower (1978) and Leong et al. (1995) also cautioned that abdominal palpation may underestimate the number of very old matings.

Multiply mated females may sometimes be mistaken for freshly mated females. The low frequency of mating and the low incidence of multiple mating in overwintering females suggests this error is not likely to be a problem at Mexican overwintering sites (Chapter 5). However, it may be a significant source of error in summer breeding monarchs due to higher numbers of matings and shorter female intermating intervals.

Just-transferred spermatophores were consistently classified as fresh for at least one week at the Mexican overwintering site. Weekly or shorter sampling intervals are therefore necessary to assess when the mass mating event begins and to describe changes in mating frequency through time using palpation. Studies of spermatophore degradation rate at different times in the overwintering period could increase palpation accuracy.

The large size and slow rate of spermatophore deterioration, together with the brief nature of the spring mass mating period and the exponential rate of increase in mating, suggests that palpation can be used to differentiate between matings that occur before versus during the spring mass mating period. However, this method is less accurate than dissection.

Female abdominal palpations can also be used to describe the timing and intensity of mass mating, to indicate changes in mating status through time, among sub-colonies, among different activities, and among years. Abdominal palpation is simple, very rapid, does

not require any equipment, can be used to assess large samples, and does not require killing the female. Furthermore, by combining palpation with counts of the number of spermatophore necks removed from the bursa opening, both the frequency of mating among females during the mass mating period and the total number of times individual females mate can be accurately determined without injuring the female.

#### Spermatophore Degradation as a Means to Differentiate Between Autumn and Overwintering Female Mating

Using both dissection and palpation, estimates of the percentage of all females with old spermatophores decreased dramatically in association with the initiation of the mass mating event in 1985, but not in 1986-1988. The 1985 finding could reflect 1) underestimates of old spermatophores either due to female remating or spermatophore degradation, 2) increased rates of spermatophore degradation and, presumably, utilization as mating activity comes into full swing, or 3) a higher death rate or an earlier remigration tendency in already mated females. The fact that dissections of the bursa copulatrix in 1985 also showed a decrease in the percent of females with old spermatophores, suggests that the first alternative is unlikely to explain my findings. Alternatives 2 and 3 suggest that a similar decrease in the percentage of females with old spermatophores should have been observed in 1986-1988 field seasons, but this was not the case.



Using only females dissected before 20 February 1985 when the mating period began, I found that 31% were already mated. Brower et al. (1977) found 15% and 23% (Brower 1985) of Mexican overwintering females were mated in January. Previous studies have generally lumped together samples across the overwintering period to estimate the frequency of mating and degree of polyandry then ascribed to the relatively brief mass mating event. My study and others (e.g. Brower 1985, Leong et al. 1995) suggest that such lumping can overestimate the importance of mating during the mass mating event. The frequency and degree of polyandry incurred in the late summer, during the southward migration, and during overwintering colony formation is expected to influence mating strategies during and after spring mass mating event and potentially influence remigration population demographics. The significance of such matings depends of the unknown viability of long-stored sperm and the fate of male-derived nutrients transferred in spermatophores. Long-term storage of viable sperm occurs in lepidopterans (Davey 1965, Parker 1970, Wiggleworth 1972, in Drummond 1984).

### Conclusions

Both palpation and bursa dissection methods gave results that were consistent with field observations of the timing and intensity the mass mating event. I discuss these results in Chapter 5. Both methods showed that 1) females mate during the southward migration and/or early on in the overwintering period, 2) the mass

mating event is restricted to the last few weeks of overwintering, increases exponentially, and most matings occur just before or just after the main exodus from the overwintering site, 3) female utilization of spermatophore contents may increase as the mating event begins, 4) many females leave the overwintering site without having mated during the mass mating event, but not necessarily as virgins, and 5) multiple mating during the mass mating period is rare, but already mated females may mate again during the mass mating period.

Knowledge of when in the life cycle female monarchs receive spermatophores is important in understanding 1) both male and female mating strategies, 2) re-migration population demographics, and 3) selective pressures that shape and maintain the migration-diapause life history strategy. Abdominal palpation and bursa copulatrix dissections provide valuable and complimentary information that can be used to increase our understanding of monarch mating ecology. These methods should be useful in studying other lepidopterans with migratory and aggregation phases.

Table 4-1. Collection dates, activity category, sample size, and percentage of combined sample of female monarchs palpated to assess mating status. Sierra Chincua, Michoacan, Mexico 1985. Successive collection periods are only approximately equal in number of days. Daily collections were combined to best represent activity areas within each collection period. Females were randomly sampled from these samples for spermatophore dissections.

Date	Flying		Drinking		Nectaring		Clustered		Total	
	n	%	n	%	n	%	n	%	n	%
1-10 Feb	126	18	268	38	101	14	211	30	706	
11 Feb-2 Mar	80	30	15	6	84	32	86	32	265	
3 Mar-15 Mar	84	15	75	13	120	22	352	56	631	
16 Mar-25 Mar	35	7	109	22	86	18	255	53	485	
Totals	325	16	467	22	391	19	904	43	2087	

Table 4-2. Collection dates, activity category, and sample size of female monarchs palpated to assess mating status. Sierra Chincua, Mexico: 1985 - 1988.

Year	Flying	Drinking	Nectaring	Clustered	Total
	n	n	n	n	n
1985	325	467	391	904	2087
1986	95	87	91	268	541
1987	56	69	20	925	1070
1988	0	0	0	1594	1594
Total	476	623	502	3691	5292

Table 4-3. Collection dates, activity category, sample size, and percentage of total combined sample of female monarchs for spermatophore dissections. Sierra Chincua, Mexico 1985. Equal numbers of palpated females from each activity category were randomly sampled and combined to form successive collection period samples. Successive sampling periods are only approximately equal in number of days.

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Collection Period	Flying		Drinking		Nectaring		Clustered		Total
	<u>n</u>	<u>%</u>	<u>n</u>	<u>%</u>	<u>n</u>	<u>%</u>	<u>n</u>	<u>%</u>	<u>n</u>
15-31 Jan	44	21	48	23	55	26	64	30	211
1-10 Feb	35	28	34	27	5	4	51	41	125
11 Feb-2 Mar	29	28	15	15	28	28	30	29	102
3-15 Mar	30	25	30	25	30	25	30	25	120
16-25 Mar	22	20	29	26	30	27	30	27	111
Total	160	24	156	24	148	22	205	31	669

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Figure 4-1. This figure was created on Adobe Photoshop, version 3.0.1, by importing 4 separate photographic images to illustrate representative examples of fresh, semi-fresh, and old spermatophores and a chitinized spermatophore neck from dissected *Danaus plexippus* females. The degree of spermatophore degradation was used to differentiate between matings assumed to have occurred during the mass mating period (fresh and semi-fresh) from those assumed to have occurred previous to the mating period (old + chitinized necks), either in the previous late summer, during the autumn migration, or early-on in overwintering colony formation. The dotted lines illustrate parts of the spermatophores that had either broken off (Semi-Fresh: the stem) or completely degraded (Neck: the entire spermatophore except for the neck). Because fresh spermatophores were distinct entities, the number of recent matings could be determined with certainty. Each spermatophore has a single chitinized neck and stem that remain after the rest of the spermatophore has degraded. These structures could therefore be used to detect the presence of completely deteriorated spermatophores and to estimate the number of matings when spermatophores were broken into indistinguishable pieces. Furthermore, because spermatophore necks extend from the bursa copulatrix opening and therefore can be used to determine the number of spermatophores without killing the female.

## Spermatophore Degradation

Fresh

Semi-Fresh



5 mm

Old

Neck (Collum)



CHAPTER 5  
TIMING AND FREQUENCY OF FEMALE MONARCH MATING  
AS RELATED TO MEXICO OVERWINTERING ECOLOGY

Introduction

Like other natural selection pressures, sexual selection arises out of competition for a limiting resource. Mate choice and intrasexual competition reflect sex differences in competition for mates. The limited sex competes for access to the limiting sex, while the latter at least potentially gains from mating selectively. Both sex differences in parental investment (Trivers 1972) and spatial and temporal distribution of individuals, or the resources they require (Emlen and Oring 1977, Clutton-Brock and Parker 1992), are important determinants of which sex is limiting and how the other sex competes for the limiting sex.

In Chapter 2 I hypothesized that large male investments in spermatophores and female nutrient need for remigration and oviposition should select for mate discrimination by both sexes based on phenotypic characters, such as age and size, that indicate the potential material benefits to be gained. I further suggested that high population densities should facilitate mate choosiness. I found evidence in support of male choice based on female size and wing condition and possibly mating status. I also found a mating advantage in small, worn males that was apparently not a result of



female choice. In Chapter 3 I showed that male mating activity was associated with old age and possibly a non-diapause reproductive state. Low reproductive gland weights, fragile tissues, depleted abdomens, and poor wing condition support an age-related male propensity to mate in Mexico.

Male mating patterns must be viewed within the ecological context of the overwintering mating event in order 1) to compare overwintering behavior of monarchs in Mexico with those in California, Australia, and summer breeders, 2) to address the reproductive costs and benefits of differences in individual mating behavior, and 3) to understand the impacts on re-colonization and population demographics.

### Monarch General Overwintering Biology

Monarch summer breeding populations range across North America into southern Canada and are associated with their host plant distribution (see Wells and Wells 1992, Malcolm et al. 1993). In the autumn, after multiple overlapping summer generations, monarchs that occur west of the Rocky mountains migrate to scores of overwintering sites along the Pacific coast of California (but see Wenner and Harris 1993), while monarchs east of the Rocky mountains migrate to a few isolated colonies in the Transverse Neovolcanic Belt in central Mexico (Brower 1985, Brower 1995). Overwintering sites are extremely small and restricted to tiny areas within montane islands of relic Oyamel fir forests, *Abies religiosa* (H. B. K. Schl. and Cham. (Pinaceae) (see references in Brower 1995).

These sites range from 0.1 to 5 ha, and are well-defined from the surrounding forest by extreme butterfly densities reported to be approximately 10 million per ha (Brower et al. 1977, Calvert in prep. in Brower 1985). All known Mexican overwintering sites occur within a geographical area of less than 3500 km<sup>2</sup>.

Monarchs arrive at the Mexican montane forest overwintering sites in November through early December and, by January, the loose aggregations become densely packed into well-delineated colonies (Brower 1985, Calvert and Brower 1986). The overwintering period lasts for approximately 5 months and co-occurs with the dry season (Brower 1985, Calvert and Lawton 1993).

Just before the northward remigration the hormonally controlled reproductive diapause is broken (Herman et al. 1989) and a mass mating activity ensues. Females leaving these few overwintering sites remigrate to the southern U.S. where they continue to mate and lay eggs (Van Hook and Zalucki 1991, Malcolm et al. 1993, Malcolm, Van Hook, Brower unpubl. data). The resulting generation continues the northward migration. Through successive generations the entire North American range, encompassing over one million square miles, is repopulated. Mid-summer populations are largest in the northern mid-western states (Malcolm et al. 1987, and references in Wells and Wells 1992).

Monarch southward migration and overwintering aggregation behaviors bring adults of broadly distributed late summer generations into close proximity before the spring remigration northward. Late summer environmental conditions, mating strategies, population dynamics, and migration behavior influence

overwintering mating ecology by affecting overwintering population size, sex ratio, age structure, and genetic and phenotypic variation. The number, timing, and quality of mating in overwintering females, in turn, may influence re-population pattern and demographics of the generations following remigration (Ehrlich and Ehrlich 1978).

### This Study

Although the general overwintering colony characteristics have been described (e.g. Brower et al. 1977, Calvert and Brower 1981, Brower 1985), no one has related colony dynamics with the timing and frequency of female mating. The purpose of this paper is to describe the timing and character of the mating event and relate these with colony characteristics. Specifically, I measured the degree and frequency of female mating with regard to colony movement, fission into sub-colonies, and the timing of butterfly dispersal from the site. I noted the importance of mating before and after the mass mating event occurs, differences in mating among sub-colonies, and changes through time and among years in 1985 - 1988. I also compared mating status among females of different ages and those collected while engaged in various activities

In 1985, bursa copulatrix dissections were performed to determine 1) the frequency of mating before and during the mass mating period and 2) the extent of multiple mating. I used the degree of spermatophore degradation as an indicator of the frequency of mating before and after the mass mating period began. In 1985 -1988 abdominal palpation was also used to estimate the

frequency and timing of female mating in live butterflies without injuring them. Dissection and palpation methods are described in Chapter 4.

I compare and contrast my findings with the results of workers who have studied monarch mating behavior at overwintering sites in California and Australia and in summer breeders. I interpret female mating patterns in terms of 1) ecological and population structure constraints on mating at the overwintering site, and 2) the potential impacts of the timing of mating and the degree of multiple mating on monarch overwintering mating tactics and on the population demographics of the first spring generation in the southern U.S.

### Methods

#### General Description of the Site

My data were gathered during the mass mating period in the Sierra Chincua colony, one of the major Mexican overwintering colonies (Calvert and Brower 1986), from 1985 through 1988. Each year the colony was located at approximately 3,000 M elevation on the south facing slope of the Sierra Chincua, located in the Transverse Neovolcanic Belt in Michoacan, Mexico, at 19°41'N, 100°18'W (Calvert and Brower 1986). In each of the 4 years, the butterflies roosted in the same ravine and often on the same trees during some part of the overwintering period. The colonies varied in size among years and through time within years and were sometimes divided

into 2 or more sub-colonies. The combined area of clustered butterflies each year was approximately 1-2 ha.

The periods of field study were 20 January - 23 March 1985, 16 February - 25 March 1986, 12 February - 7 March 1987, and 18 February - 22 March 1988. In 1987 I also obtained a late season sample from 29 March, collected by Alfonso Alonso-M. and Alfredo Arellano-G.

Activities are limited during much of the overwintering period by cold temperatures. Beginning in early February activities, such as flying, nectaring, drinking, and mating, increase dramatically with the warming temperatures. These activities are generally restricted to within the roosting site where butterflies that had been active re-cluster in the late afternoons. By middle February the colony or sub-colonies may move short distances downhill and usually fragments into 2 or more sub-colonies. Mass mating usually begins in middle February and builds in intensity until the colony breaks up in middle March to late March, when the butterflies begin their remigration northward. Butterflies may depart in a series of mass exits, or the colony may gradually dwindle in size as they leave.

#### Wing Condition Rankings

Using wing condition rankings as an indicator, I compared female mating status versus age both before and after the mass mating period began on 20 February 1985. I ranked wing condition as good, medium or poor. The parameters I used included 1) degree of scale fading, 2) scale loss, 3) scratches, and 4) tattering (fraying) of

wing margins. Each butterfly was held up to a light source or the sun while holding the wings spread open so that both the upper and lower surfaces could be inspected. Representative examples of good, medium and poor wing condition rankings are shown in Chapter 2 (Figure 2-2).

Because my objective was to use wing condition to estimate physical age of monarchs rather than distance traveled or experiences incurred, I attempted to ignore damage such as beak marks, missing wing area believed to be attributable to bird damage, or damage due to net capture or handling, including thumb and net prints.

#### Bursa Copulatrix Dissections

Bursa copulatrix dissection methods are described in Chapter 4. I dissected the bursae of 669 females collected between 15 January and 25 March 1985. I randomly sub-sampled equal numbers of clustered, flying, nectaring, and drinking females from the daily samples that I had palpated (see below) so as to obtain approximately equal representation among activity categories. This was done both to get a representative sample of overwintering females and to assess possible differences in mating status among females engaged in different activities. However, because all butterflies roost at night and their exit from clusters during the day is dependent on attaining flight threshold body temperatures (Masters 1993), and because flight is required to reach water and nectar sources, these activity categories are not mutually exclusive.

Overall, 31% of dissected females were clustered, 24% were flying, 22% were nectaring, and 23% were drinking. Collection dates, activity category, sample size and percentage of the combined weekly dissection samples collected from each activity category are given in Chapter 4 (Table 4-3).

I classified spermatophores as fresh, semi-fresh or old. These classifications were used to differentiate matings assumed to have occurred during the spring mass mating period (fresh and semi-fresh) from matings assumed to have occurred previously during the late summer, during the autumn migration, or during the overwintering colony formation. Dissections of females that were collected *in copula* (unpubl. data), changes in spermatophore mass as detected by palpation (Chapter 4), and dissections of spring remigrants and summer breeders support these designations (see also Svärd and Wiklund 1988a, Oberhauser 1992).

#### Female Abdominal Palpations

Males transfer a large spermatophore to females during mating so that the mating status of live females can be assessed by gently applying pressure between the thumb and index finger while feeling along the abdomen from anterior to posterior for a hardened mass in the bursa copulatrix (Tuskes and Brower 1978). I categorized females as virgin, mated, or freshly mated based on the relative size and hardness of the spermatophore inside the bursa.

I palpated a total of 3205 females collected between 1 February and 25 March 1985; 43% were clustered, 16% flying, 19%

nectaring, and 22% drinking. Sample sizes of clustered females were always larger than those from other activity categories because clustered females were collected in mass while females in all other activity categories were netted singly. Collection dates, activity category, sample size, and the percent of the combined weekly samples collected from each activity category are given in Chapter 4 (Table 4-1).

In 1986, 1987, and 1988 I palpated a total of 2690 females between mid-February and late March. In 1986 I used the same activity categories as I did in 1985, but in 1987 I concentrated on collections from tree clusters, and in 1988 I collected only from tree clusters. Sample sizes among activity categories for 1985-1988 are given in Chapter 4 (Table 4-2).

## Results

### Overwintering Colony Characteristics

Between early February and late March from 1985 through 1988 I observed from 1 to 5 sub-colonies at the Sierra Chincua overwintering site. Sub-colonies were defined by clustered monarchs roosting at night. They varied in the time of formation, size, longevity, mobility, and in the level of butterfly mating activity within the sub-colony. Later forming sub-colonies generally formed downhill from existing sub-colonies. Dispersal from the overwintering site began in middle to late March. Only in 1986 did a



large proportion of overwintering monarchs remain in the area at the end of the observation period.

The colony, or sub-colonies, moved downhill through time following stream beds dominated by oyamel fir trees. The timing and number of movements were associated with sharp increases in mating frequency. Two kinds of movement occurred: 1) small daily changes in colony boundaries such that over several days or weeks the butterfly aggregation gradually moved downhill, or 2) distinct downhill jumps of a whole sub-colony with intermediate areas never being utilized for roosting. No jump was more than a few hundred meters downhill and sub-colonies were separated by a similar distance. In 1985 and 1988 a single large colony predominated throughout the aggregation period, moving both gradually and in jumps downhill, but in 1986 and 1987 multiple sub-colonies occurred simultaneously.

A substantial number of monarchs left and returned to roosting sites during warm and sunny days, but in the morning and evening and during inclement weather butterflies remained clustered throughout the day. Butterfly flight, mating, drinking and nectaring were generally restricted to within the distinct colony boundaries. As spring progressed toward the end of March, more and more butterflies could be found drinking and nectaring outside, and usually below, the colony area. Although mating and post-nuptial flight (see Chapter 2) were generally restricted to within the colony, very late in the overwintering period, mating also occurred at nectaring sources that were extensive enough to attract large number of butterflies.

Consistently among years, the sex ratio became more female-biased at the end of the overwintering period. This change in sex ratio may be partly due to differential mortality of males. Brower and Calvert (1985) and Alonso-M. (1996) showed that males were more likely to be killed by birds than females at the overwintering site. However, bird predation accounts for only a small loss of the overall population and the sex bias was not extreme. Males are also the first to break reproductive diapause. Because breaking diapause is associated with an increased rate of senescence, males may die sooner than females. However, in California, where males break diapause earlier than in Mexico, the late overwintering sex ratio is extremely male, rather than female, biased (Hill et al. 1976, Tuskes and Brower 1978, Wells and Wells 1992).

Because males 1) are the first to break diapause, 2) are the first to move downhill as colonies become more dynamic late in the overwintering period, and 3) they occur in approximately equal proportions as females in spring remigrants collected in the southern U.S. (Knight and Brower in prep., Chapter 3), I suggest that the bias toward females at the end of the Mexico overwintering season is primarily because males are dispersing first.

#### Yearly descriptions of colony movement and sub-colony formation

Approximate dates of formation, departure, relative size, and relative uphill-downhill position of the sub-colonies observed during the overwintering observation periods from 1985 through 1988 are shown in Figure 5-1. The approximate date that mating was first

observed and the mass mating period began are also indicated. The increasingly dynamic nature of colonies, the similar timing of the initiation of mating activity, and the association between mating and colony movement can also be seen in Figure 5-1.

In 1985 a single colony remained stationary from when my observations began in early January until 25 February when it fissioned and a sub-group jumped downhill approximately 100 m forming a smaller sub-colony. Over the next week the remaining uphill sub-colony members joined the downhill group re-establishing a single colony which remained stationary through the end of March when the colony broke up. Visual estimates indicated that only about 10% of the colony remained in the area on 25 March.

In 1986 there were 3 sub-colonies when I arrived at the site in mid-February. The most downhill one remained consolidated through the rest of the observation period, moving gradually downhill through time. Butterflies in the 2 more uphill sub-colonies left their initial sites by late February and, presumably, joined the downhill sub-colony. Early in March a fourth sub-colony fragmented from this reunited colony. This relatively small aggregation apparently jumped farther downhill, when in mid-March, a fifth, most downhill sub-colony appeared. This sub-colony first dwindled, then grew in late March, presumably by recruitment from the remaining larger uphill sub-colony, but perhaps also from butterflies coming in from more distant overwintering colonies. Only small numbers in the original colony and the most late-formed aggregation site remained in the area at the end of March.

In 1987 there was a single large colony when I arrived in early February. On 11 February the entire colony jumped downhill. The most substantial number of monarchs remained in this position through the end of March. On 28 February the colony apparently fissioned forming a second, smaller, more downhill sub-colony. In early March a third, even smaller sub-colony was formed uphill from the second sub-colony. This was the only time during my 4 years of observations that a sub-colony formed uphill from an existing colony. However, recruitment into the new sub-colony probably came from the remaining initial large aggregation that was still positioned uphill from the new site (see Figure 5-1).

The 1988 colony dynamics were similar to 1985. A single large colony fissioned into 2 sub-colonies in mid-February when a subgroup of the original colony jumped downhill. Within about 10 days butterflies remaining in the initial site consolidated with the downhill sub-colony to re-form a single colony in the more downhill position. This large colony again jumped downhill in mid-March, but moved from the stream bed it was following to another ravine about 0.5 km to the west. The area where the butterflies re-positioned was unlike all the other roosting sites observed. It was situated in a completely dry ravine with open stands of pines. Even though the known pre-existing site was completely devoid of monarchs, this colony continued to grow, rearrange, and consolidate in mid-March. This growth may have resulted from monarchs coming in from other more distant colonies. I also noted thousands of monarchs flying over my campsite, located on the mountain ridge approximately 1 km to the south of the colony site on the 12th and 13th of March.

Also, another known overwintering site, Palomas had completely dispersed on the 16th of March. Some of these butterflies may have joined the Sierra Chincua colony. This latest-formed colony dispersed abruptly on the 22nd of March with only about 10% remaining the following day.

### Fire and colony dynamics

The monarch overwintering period coincides with the dry season (Brower 1985, Calvert and Lawton 1993). During my 4 field seasons at the Sierra Chincua overwintering area, I occasionally viewed fires from my campsite located within 1 km of the butterfly colony. On two occasions, once in 1987 and once in 1988, a fire was large and close enough to affect the behavior of the butterflies. During both of these fires, except for the butterflies roosting on a few densely clustered trees and scattered leaf clusters, virtually all the butterflies in the colony became active. Whole clusters of monarchs throughout the colony were seen cascading simultaneously off the boughs and taking flight. Flight was erratic and restricted to the colony area. Clusters reformed in the evening immediately below the pre-existing colony area, and the colony boundaries lengthened along the uphill-downhill axis. Butterfly density within the colony decreased. Over a few days, in 1988, but not 1987, the colony appeared to re-consolidate to a density similar to that seen just before the fire.

The effect of the distant fire on the form of the colony was extremely dramatic in 1987. From my campsite the frenzied colony

was defined against the forest canopy by the orange wings as the butterflies flew in dense swirls just above the green treetops (see photograph by Glendinning, in Malcolm and Zalucki 1993). Butterfly flight resembled swarming bees. How distant fires might effect lipid depletion, predation exposure, mating dynamics, or overwintering success is not known, but certainly these two fires were associated with at least temporary changes in the structure and dynamics of the overwintering colony.

### Mating Activity

The timing of the initiation of mating and the timing of the build up to mass mating varied little among years. The date of the first observation of mass mating activity ranged from 11 February in 1987 to 20 February in 1985. Mating began to dominate butterfly activity on the 3rd of March in 1985, 1986, and 1987 but not until 14 March in 1988 (see Figure 5-1). The later date in 1988 reflects the unusually cold and overcast weather in early March of that year. Maximum temperatures remained under 8 ° C until the 12th of March after an unusually late snow storm occurred on the 4th of March.

Unlike the timing, the intensity of the mass mating event varied among years and appeared to reflect weather conditions. Mating activity, and activity in general, was greatly reduced on cold and overcast days. For example, although mating began relatively early in 1987, unusually cold and overcast weather during most of the rest of the overwintering period kept overall frequency of

mating low that year. Field observations indicated that mating activity, but not other activities, remained depressed under warm and sunny conditions for a few days after periods of unusually cold weather.

In all years, mating activity increased slowly then dramatically just before colony breakup. Abrupt increases in mating activity were generally, but not always, associated with increases in daily high-low temperatures and colony fission and movement. Mating was more common in the late afternoon and its frequency dropped off consistently and dramatically outside of the colony boundaries.

In all years, field observations of the timing of the initiation and build up of mass mating were confirmed by female abdominal palpations. For example, in 1985, field observations, abdominal palpations, and bursa copulatrix dissections all indicated that mass mating began between 16 and 20 February.

Dramatic increases of mating activity were associated with downhill movement and fission of the overwintering colony into distinct sub-colonies (see Figure 5-1). Colony movement and increased mating activity were both associated with warming weather, but in 1988 the initiation of mating and sub-colony fission occurred simultaneously and in the absence of any sudden change in weather conditions.

Field observations indicated that mating was generally more common in the downhill rather than uphill sub-colonies (see Figure 5-1). Temperature differences did not appear to account for this difference. In 1986, between 11 March and 25 March, during the peak of mass mating, I compared the rate of male-female pre-

copulatory pairs that fell to the ground within 30 minutes in a 3 X 3 m arena positioned in a sunny location in the center of an upper and lower sub-colony. Six upper and lower samples were matched for time of day, cloud cover, and temperature. The results are shown in Figure 5-2. Consistently and dramatically more attempted copulations were observed in the lower sub-colony (Wilcoxon signed rank test:  $Z = 2.20$ ,  $p = 0.03$ ,  $n = 6$ ).

I also compared the percentage of mated females, as determined by palpation, in 8 samples collected from uphill and downhill sub-colonies matched for the date of collection. These samples were chosen from all population census samples from 1986 through 1988 based on the presence of 2 simultaneous sub-colonies in which data were collected from both within a few days' period. Sex ratios and percentage of mated females therefore vary greatly among sample pairs, due to changes in mating frequency through time, but the trend of a higher proportion of mated females in the matched downhill sub-colony is evident across all sampling dates (Table 5-1). Both the percentage of mated and freshly mated females were higher in downhill sub-colonies (Wilcoxon signed rank test:  $Z = 2.52$ ,  $p = 0.01$ ,  $n = 8$ ;  $Z = 2.10$ ,  $p = 0.04$ ,  $n = 8$ , respectively). The sex ratio was significantly more male-biased in the lower than the upper sub-colonies ( $Z = 2.38$ ,  $p = 0.02$ ,  $n = 8$ ).

#### Frequency of Female Mating

Of all overwintering females dissected in 1985, 67% ( $n = 669$ ) were virgins. Of the 33% that had mated, 69% had only old



spermatophores and presumably had mated in the late summer, during the autumn migration, or early on in the overwintering period, 29% had only fresh or semi-fresh spermatophores obtained during mass mating, and 2% had both old and fresh spermatophores. These latter females presumably were already mated when the mating event began and remated during that time. When only females sampled before 20 February, when mating became prevalent, are considered 31% had previously mated. Their spermatophores were almost all extremely degraded.

Female mating during the mass mating event was surprisingly rare. If only females collected during the mass mating period (20 February - 25 March) are considered, the percentage of females with fresh or semi-fresh spermatophores was only 20% ( $n = 290$ ).

Multiple mating was also rare. Only 7% of all dissected female were multiply mated. These accounted for 21% of all mated females. Eighty-three percent of these had only old spermatophores suggesting that, by far, most polyandry reflected matings that occurred prior to the mass mating event. Nine percent of multiply mated females had both old and fresh spermatophores, suggesting some remating of previously mated females during the spring mating event. Only 9% of multiply mated females obtained all their spermatophores during the mating period. As indicated by dissection, there was no evidence that previously mated females were more likely to mate than virgins, in fact the trend was in the opposite direction. Twelve percent of virgins mated during the mass mating period in comparison with 3% of non-virgins.

The mean wet weight of fresh spermatophores was  $18 \pm 4$  mg ( $\pm$ SE; range 7-120 mg,  $n = 31$ ), while the average wet weight of semi-fresh spermatophores was  $6 \pm 1$  mg (mean  $\pm$  SE; range 2-17 mg,  $n = 25$ ). The mean fresh spermatophore wet weight from wild-caught overwintering females was almost identical to that of females known to have just received a spermatophore ( $16.4 \pm 1$  SE,  $n = 30$ , see Chapter 4), and was within the range found for wild-caught summer breeders (Oberhauser 1992). Old spermatophores were not weighed due to their often extremely deteriorated and fragmented condition.

The mating status of overwintering females in 1985 is contrasted with remigrating females collected in the early spring of 1985 in the southern U.S. in Table 5-2. Cardenolide thin-layer chromatography patterns were used to separate remigrating females from first generation females produced in the U.S. (see Malcolm and Brower 1989, Malcolm et al. 1993). Combining fresh, semi-fresh and old spermatophore count totals from 669 bursa copulatrix dissections of overwintering females collected between 15 January and 25 March, 67% were virgins, 26% were mated once, 3% twice, 0.1% 4 times, and 4% were mated an unknown number of times. These latter females were those with spermatophore parts that were too disintegrated to distinguish distinct matings. Overall, only 7% of overwintering females were multiply mated ( $n = 669$ ). In contrast to the overwintering butterflies, 100% of remigrating females were mated from 1 to 8 times. Ninety percent were multiply mated, with 46% mated 4 or more times ( $n = 48$ ) (see Table 5-2).

Changes in female mating status through time: Bursa copulatrix  
dissection: 1985

Changes in female mating status through time, as estimated by bursa copulatrix dissection, are shown in Figure 5-3. There was a sharp decrease in the percentage of females with old spermatophores detectable by dissection associated with the beginning of the mass mating period. Assuming that even very old matings are detectable by dissection, the percentage of females with old spermatophores should not decrease through the overwintering period. As spermatophores deteriorate through time their detection requires finding the chitinized necks. The measured decrease in the percentage of females with old spermatophores is probably partly an artifact created by not always being able to detect chitinized necks using the dissection techniques. This error can be reduced by using wet dissection techniques in which the contents of the bursa copulatrix are sonicated and then inspected under a dissecting microscope (see Chapter 4). The decrease may also indicate an association between prior mating and death at the overwintering site or differential exodus of previously mated females. However, such a decrease did not occur in any other year (see Figure 5-4, below).

Mating frequency grew exponentially during the approximately 6 week long mating period. Less than 1% of all females dissected in January ( $n = 211$ ) had fresh or semi-fresh spermatophores in their bursa, confirming that mating had not yet begun. The percent of females with fresh or semi-fresh spermatophores increased to 17% in early March (3 - 15 March:  $n = 120$ ), and 33% by late March (16 - 25

March:  $n = 111$ ) (see Figure 5-3). If only females collected on the last 3 days of the observation period are included (23 - 25 March), the percentage of freshly mated females jumps to 39% ( $n = 51$ ). Furthermore, of all the matings determined to occur during the mass mating event, 45% occurred during the last week. Although female mating increases exponentially, a large percentage apparently began remigration without mating at the overwintering site (see Figure 5-3).

Mass mating at Mexico overwintering sites may be a limited source of female mating compared to mating during the late summer, the autumn migration, or the early overwintering period. There was no significant change in the occurrence of mated versus virgin females ( $X^2 = 0.44$ ,  $df = 1$ ,  $p = 0.51$ ,  $n = 699$ ), or the frequency of multiple mating before and after mass mating began ( $X^2 = 1.13$ ,  $df = 1$ ,  $p = 0.29$ ,  $n = 669$ ). As expected, the relative frequency of recent matings did significantly increase after mass mating began ( $X^2 = 50.9$ ,  $df = 1$ ,  $p = < 0.0001$ ,  $n = 669$ ).

Changes in female mating status through time: Abdominal palpation: 1985-1988

Females collected early (15 - 29 February), middle (1 - 12 March), and late (13 - 29 March) in the mating period from 1985 through 1988 were categorized by abdominal palpation as having mated before (old spermatophores) or during (fresh + semi-fresh spermatophores) the mating period, or as virgins. The timing and relative frequency of female mating among the 4 consecutive overwintering mating periods is shown in Figure 5-4.

The percentage of overwintering females with old spermatophores before the mass mating period ranged from 17 to 38%. Mating by females before or during the southward migration to Mexico, or early on in the overwintering period is variable, but relatively common in monarchs. In 1985 abdominal palpation results were consistent with dissection results in showing a decrease in the percentage of females with old spermatophores through time. However, this trend was absent in 1986 through 1988.

In all years mating frequency began building slowly in middle to late February, when colonies began fissioning and mating became frequently observed in the field. Between 4 and 15% of females became freshly mated during this time. In every year except 1987, when the weather was unusually cold in March, mating frequency increased most dramatically just before remigration began. By late March, just before remigration, the percentage of freshly mated females ranged from 14% in 1987 to 35% in 1986.

Consistent with dissection results, in all 4 years a large proportion of females apparently left the overwintering site without mating during the mass mating period. Forty to 67% of the females remained virgins through the late March sampling period. The frequency of mating at Mexican overwintering sites varies considerably and probably largely reflects weather conditions. In years with unusually cold or overcast conditions (e.g., 1987) very few females may mate before beginning the northward migration.

### Female Activity Versus Mating Status

In 1985, using abdominal palpations, female mating status (mated versus virgin) varied among activity categories before mass mating began on 20 February ( $X^2 = 17.18$ ,  $df = 3$ ,  $p = 0.006$ ,  $n = 861$ ). The proportion of mated females by activity category was, in order from lowest to highest, 34% drinking, 41% clustered, 46% nectaring, and 53% flying. Tukey-type multiple comparisons of the proportions of mated females among the different activities indicated that drinking females differed significantly from nectaring and flying females but not from clustered females, while clustered females were not different any other group (see Table 5-3). The presence of large amounts of water in the abdomen of drinking females may have made detection of deteriorated spermatophores by palpation more difficult. During the mass mating period, the percent of freshly mated females ranged from 14% (clustered) to 19% (nectaring), but did not differ significantly among the 4 activity categories. ( $X^2 = 4.20$ ,  $df = 3$ ,  $p = 0.24$ ,  $n = 1226$ ). All 4 categories were therefore combined to test for differences in mating status among females of varying wing condition (see below).

Unlike 1985, in 1986 during the mass mating period there was a significant difference in the frequency of freshly mated females versus those classified as either virgins or with old spermatophores among activity groups ( $X^2 = 16.7$ ,  $p = 0.0008$ ,  $df = 3$ ,  $n = 541$ ). Forty-three percent of nectaring females had fresh spermatophores whereas drinking, clustered and flying females ranged from 18 to 26% mated percent freshly mated. Tukey-type multiple comparisons

of the proportion of freshly mated females among activity groups showed that nectaring females differed significantly (at  $p = 0.05$ ) from all other categories, while clustered, drinking and active females did not differ (see Table 5-3).

### Female Condition Versus Mating Status

Using wing condition as an indication of age, I asked whether older females were more likely than young females to mate during the autumn migration or at the overwintering site before mass mating began. Using combined samples of all activity groups collected before 20 February 1985 when mass mating began, I compared wing condition (good, medium, poor) between mated and virgin females, as determined by abdominal palpation ( $X^2 = 22.5$ ,  $df = 2$ ,  $p = 0.0001$ ,  $n = 861$ ). Females in worse condition were more likely to have mated before the mass mating period (see Figure 5-5a).

Females found *in copula* during the mass mating period were in better wing condition than females in the general overwintering population (Chapter 2). If good condition females are more likely to become mated during the mass mating event, then female mating status, as determined by palpation, should differ among good, medium and poor condition females. Females mated during the mass mating period (fresh + semi-fresh spermatophores) differed from those that did not (old spermatophores + virgins) in wing condition at  $p = 0.06$  ( $X^2 = 5.74$ ,  $df = 2$ ,  $n = 1226$ ), but the trend was in the opposite direction of that predicted by male mate choice: A

relatively higher percentage of females in poor condition were palpated as freshly mated (see Figure 5-5b).

## Discussion

### Overwintering Colony Characteristics

Monarch butterfly overwintering ecology and population structure potentially influence the number, timing and quality of female matings through opportunities and constraints on the mating frequency, mate choice, sperm competition, lipid expenditures, and possibly transfer of nutrients to females via spermatophores. Female mating patterns in turn potentially influence remigration potential and the evolution of the monarchs migration-overwintering-mass mating life history strategies (Brower 1985, Tuskes and Brower 1978, Wells et al. 1993, but see Svård and Wiklund 1988a).

The Sierra Chincua overwintering colony position, fission and downhill movement were generally consistent among the 4 years of observation and similar to earlier accounts (Urquhart and Urquhart 1976, Brower et al. 1977, Brower 1985, Calvert and Brower 1986). I observed variation among years in the timing of sub-colony formation and sub-colony number, size, longevity, mobility, cohesiveness, and level of mating activity. In 1988 a sub-colony that appeared in late March apparently grew from recruitment from other distant colonies. Recruitments have been suggested for Mexican overwintering areas by Calvert and Lawton (1993) and is



implied at California sites (see Tuskes and Brower 1978, Leong et al. 1993).

Colonies became increasingly dynamic through time. Fission into sub-colonies and downhill movement was 1) associated with but not dependent on changes from cold and overcast, to sunny and warm weather conditions and 2) associated with, but not necessary for, initiation of and dramatic increases in mating activity. Sub-colony fission and movement may play a role in boosting mating activity in Mexico overwintering colonies. Calvert and Lawton (1993) also noted that overwintering colonies become more dynamic around the time that mass mating begins. They suggested that downhill movement and re-positioning functions to maintain appropriate micro-climatic conditions, especially water availability. My observations of a close tie between movement and mating even in the absence of changes in weather conditions, and especially the 1988 late season formation of a sub-colony in an unusually open and dry area, suggest that mating may be a concurrent pressure for colony movement. Monarchs may be seeking sunshine and warmer temperatures at this time, unlike during most of the overwintering period. California overwintering sites are noted for becoming increasingly dynamic toward the end of the overwintering period (Wells and Wells 1992), but reports of colony fission and repositioning in the immediate area are lacking.

Dispersal from overwintering sites began in middle to late March and occurred either as a series of mass exodus or, less commonly, butterfly numbers dwindled more or less steadily. Colony dispersal appeared generally compacted in time compared to more variable

windows of dispersal described for California overwintering sites (Downes, in Williams et al. 1942, Hill et al. 1976, Tuskes and Brower 1978, Leong et al. 1993) and Australia (James 1993).

Apparently in response to smoke associated with 2 separate large fires, I observed almost the entire overwintering population cascade from their roosting sites and fly frantically around the colony area. This activity was uniquely associated with the nearby fires, was apparently energetically costly, and is a conservation concern (Brower and Malcolm 1991). On both occasions the colony reformed slightly downhill, with an increase in colony area and a decrease in butterfly density. Fire-caused energetic costs to individuals, and changes in population dynamics could influence both the mating ecology and survival of much overwintering in Mexico.

The most dramatic difference observed between the Mexican and California overwintering colony dynamics (Downes, in Williams et al. 1942, Hill et al. 1976, Tuskes and Brower 1978, Frey and Leong 1993, Leong et al. 1993, Nagano et al. 1993) was an opposite trend in sex ratio bias at the end of the overwintering period. In my study (see Table 5-1; also see Chapter 2) the sex ratio became increasingly female-biased, whereas California researchers consistently document the opposite. This late season male-bias also occurs in Australia, but appears much less dramatic than in California, except in transient overwintering populations which are consistently highly male-biased (see James 1981, James 1982a, James 1984b).

### Mating Activity

Consistent among years and as previously observed by Brower (1985) and Calvert and Brower (1986), mass mating began in mid-February and increased exponentially until colony breakup in mid-to late March. The mass mating event appears generally more prolonged in California. The onset of mating occurs earlier, usually in January, and dispersal dates are more variable, ranging from January (Leong et al. 1995) to early May (Downes, in Williams 1942). Earlier mating and sometimes earlier exiting probably reflects the more moderate spring temperatures characteristic of the California overwintering areas. Mass mating has not been reported at Australia overwintering sites (James 1993).

The intensity of the mass mating event in Mexico varied among overwintering seasons, apparently reflecting differences in weather conditions. The interaction of environmental cues that influence the date of onset and intensity of mating activity and those that initiate dispersal from the sites may determine the relative frequency of mating at overwintering sites may reflect .

Mating activity was dramatically depressed during cold weather and somewhat depressed during overcast conditions. In agreement with reports of California overwintering monarchs (Hill et al. 1976), mating activity was apparently boosted by warm, sunny weather of the immediately preceding days. Mating sometimes remained depressed on warm, sunny days immediately following long spells of unusually cold and overcast weather. Also in agreement with reports of California overwintering monarchs (Wells

and Wells 1992, Leong et al. 1995), mating frequency dropped off dramatically outside of colony boundaries. When sub-colonies co-occurred, mating was more common in the downhill sub-colonies. These were also more male-biased than uphill sub-colonies.

In contrast to observations of peak mating during mid-morning at the California overwintering sites (Wells et al. 1990, Leong et al. 1995), but similar to observations of summer breeders (Oberhauser 1992, Svård and Wiklund 1988b, pers. obs.), mating in Mexico was more common in the late afternoon. This occurred even when temperatures were conducive to flight early in the day. My observations do not support the suggestion by Wells and Wells (1992), that high population densities at overwintering sites result in most matings occurring early in the day.

Monarch mating behavior was not noticeably different from reports of overwintering monarchs in California (Hill et al. 1976) or summer breeders (Zalucki and Kitching 1982, Oberhauser 1988), with the exception of Leong et al. (1995) who reported many aerial males capturing females that were inactively sunning in the canopy. I saw a male attempt to capture a perched female only once in 4 years. Mate pairing success rates observed during the ground phase pre-copulatory interactions (see Chapter 2) appeared relatively low compared to summer breeders (pers. obs.).

#### Female Mating Before the Mass Mating Event

Considering only females sampled before the mass mating event began in middle February 1985, an estimated 31% of females

were mated. The percentage of palpated females with old spermatophores ranged from as little as 17% to as high as 38% early in the mating period. These results are consistent with previous estimates of mating frequency in pre-mass mating overwintering females (17 to 22% (Herman et al. 1989, Brower et al. 1977, Brower 1985)), and suggest that females mate in the late summer, during the autumn migration, or early in the overwintering period. Mating during the autumn migration and/or during overwintering colony formation may be even higher in California (e.g., 33% (Downes, in Williams 1942), 15 to 33% (Tuskes and Brower 1978), 29% (Herman et al. 1989), and 51% (Leong et al. 1995), and even higher in Australia (greater than 70%, James 1993)).

Mating has been observed at temporary aggregation sites during the autumn migration: 29% along the Gulf coast (Brower 1985, pers. obs.), and 5 to 9% in Texas (Brower 1985). Also, along the southward migration, some females are reproductive as evidenced by oviposition, at least in the southern U.S. (Downes, in Williams 1942, Brower pers. comm., pers. obs.). Mating by females before arriving to California overwintering sites has also been reported (Downes, in Williams 1942, Ackery and Vane-Wright 1984).

Low levels of mating during colony formation and throughout the overwintering period have also been reported in Mexico (Urquhart and Urquhart 1976, Brower et al. 1977, Brower 1985), California (Downes, in Williams et al. 1942, Urquhart 1960, Hill et al. 1976, Tuskes and Brower 1978, Leong et al. 1995) and Australia (James 1984b).

### Frequency of Female Mating During the Mass Mating Period

Although the levels of pre-mass mating are apparently similar in California and Mexican overwintering sites, the level of female mating during the mass mating period is much lower in Mexico. Bursa copulatrix dissections from 1985 indicated that overall 33% were mated and 67% were virgins. Only 20% of all females collected during the mass mating period had recently mated. Using abdominal palpation as an indicator, the frequency of female mating varied considerably among years, apparently due to differences in weather. From 1985 through 1988, late March (15 - 29 March) estimates ranged from 13 to 35%.

Female mating frequency increased exponentially throughout the approximately 6 week long mass mating event. In 1985 I found that 45% of all matings that occurred over the 6 week mass mating event occurred during the final week. Considering only females collected during this final week, the percentage of freshly mated females was 39%. This compares with 17% in early March. However, the intensity of mating activity varied among years and was associated with warm and sunny conditions. California overwintering monarchs also show an exponential increase in mating activity, and most matings occur during the last few weeks of the overwintering period (Downes, in Williams et al. 1942, Hill et al. 1976, Tuskes and Brower 1978, Leong et al. 1995).

Hill et al. (1976), Tuskes and Brower (1978), Herman et al. (1989), and Leong et al. (1995) all report near universal mating in California late overwintering females. This difference probably

partly reflects differences in sampling methods. The extremely high California estimates were made at the very end of the overwintering period, often after the largest portion of the population had already exited, and during which time sex ratios were extremely male-biased. My considerably lower estimates come from samples taken regularly throughout the mating period. During the first few weeks of this time, although mating is commonly seen in the field, only a small portion of the females were mated. However, even in late season samples, mating appears to be much less frequent than at California overwintering sites. Interestingly, in Australia overwintering monarchs, where mass mating does not occur, most females are mated soon after colony formation, and virgins never account for more than 30% of females (James 1993).

Between 65 and 86% of females dispersed from the Sierra Chincua overwintering site without mating during the mass mating event. Consistent with my findings, Brower (1985) estimated 70% and Herman et al. (1989) estimated 85% of females left Mexico overwintering sites without mating. This high percentage suggests that mating is apparently not the cue for female exodus from Mexican sites, as suggested for California (Tuskes and Brower 1978). Many dispersing females may not be virgins, however, due to the commonness of mating before the mass mating period. Furthermore, the time remigrating females reach the southern U.S. 100% are mated. This indicates that mating after colony breakup and/or along the return migration is common in females that overwinter in Mexico.

### Polyandry

The overall frequency and degree of multiple mating was low at the Mexican overwintering site. Only 7% of all dissected females, or 21% of mated females were multiply mated. Brower et al. (1977) found 25% of mated females were multiply mated in January of that year. In California overwintering females, Leong et al. (1995) found 33% of all females, or 54% of mated females were multiply mated.

Including those mated an unknown number of times, I estimated 11% of mated females were mated more than twice in Mexico. Dissections or stem counts from other years are necessary to determine if the low incidence of polyandry seen in 1985 is anomalous or general for Mexico overwintering females. For comparison, Brower (1985) found 8% of mated females in Mexico were mated more than twice, with a maximum of 4 matings. Leong et al. (1995) did not report the degree of polyandry in their study, but combining females that were mated more than twice, they found 15% had been mated 3 or more times. The highest number of matings reported from California females is 7 (Hill et al. 1976).

The mass mating at the end of the overwintering season was apparently not the source of most multiple matings. In 1985, 83% of all polyandrous females were multiply mated before the mass mating event and did not remate during this time. Only 9% of virgins mated multiple times during the mass mating event. The relative frequency of polyandry in pre-mass mating females (25% of all mated females) was comparable to that seen in California



overwintering females collected before mass mating (30%; calculated from Leong et al. (1995) October - December samples).

While the frequency and degree of multiple mating by individual females appear to be comparable during colony formation, late in the overwintering period they are much lower in Mexico than in California (Downes, in Williams et al. 1942, Hill et al. 1976, Leong et al. 1995). The proportion of females with fresh spermatophores increased during the mass mating event in Mexico, but there was no associated increase in the frequency of polyandry from that measured before mating began (25% versus 15%). Leong et al. (1995) found that multiple mating increased dramatically by the end of the overwintering period to 80%. These California data, but not my Mexico data, support the prediction by Wells et al. (1993) that the number of spermatophores per female should increase as the frequency of matings increase due to the highly aggregated reproductive population (wells et al. 1993).

Using bursa dissections as an indicator, I found no evidence that already mated females, assumed to be in greater need of resources due to their poor wing condition and nectaring behavior, sought out matings to replenish depleted nutrients. Only 3% of non-virgins remated during the mass mating event, while 12% of virgins mated. Both Downes, (in Williams et al. 1942) and Leong et al. (1995), report female remating during the mass mating event, and Downes suggested these may be necessary to replenish sperm and/or nutrients in California overwintering females. However, the higher percentage of both old and new matings associated with female age and nectaring behavior found in my study (see below) suggests that

female needs may influence their likelihood of mating either directly, by seeking out copulations, or indirectly, by seeking nectar and increasing exposure to sexually active males. Regardless of female nutrient needs, and in contrast to suggestions by previous workers (e.g. Herman 1981, Brower 1985, Wells et al. 1993), the overall low level and degree of multiple mating at the Mexico overwintering site offers negligible refueling resources for remigrating females.

Both Mexico and California late overwintering polyandrous females apparently carry a combination of spermatophores from copulations obtained before leaving summer sites, during the autumn migration, early in the overwintering period, and during the mass mating period. However, the relative proportions obtained during the different summer breeding-migration-overwintering-mass mating phases of the monarch's life history cycle apparently vary between these eastern and western U.S. populations. Assuming continued sperm viability, such variance is expected to influence mating strategies, repopulation dynamics, and perhaps overwintering and remigration potential.

The frequency and degree of polyandry at the Mexican overwintering site is also in contrast with that found in females collected in the southeastern U.S. during the spring remigration of the same year. There, all females were mated, 90% were multiply mated up to 8 times, and 46% were mated 4 or more times. The high degrees of polyandry found in spring remigrators, at least in the southeastern U.S. suggest that mating along the remigration is common. Such matings may boost female flight fuels. However, the low concentrations of lipids and small size of spermatophores suggest

that even the degree of multiple mating observed in remigrating females may be limited as a source of flight fuels. Because spermatophores contain proteins not available in nectar, remating during the remigration may be more important for egg production than fuel for flight.

Furthermore, at least some of these females apparently carry sperm from matings that occurred 6 to 9 months prior to remigration. Because mating is relatively rare in Mexico, assuming that long-stored sperm remains viable, it is likely that at least a percentage of the first generation offspring in the extreme southern U.S. are sired from matings that occurred before remigration. Although monarchs show a strong last male advantage in sperm competition, sperm precedence patterns are variable and influenced by the time between matings (Oberhauser pers. comm.). We therefore cannot assume that successfully remigrating males are the exclusive sires of the first generation produced in the southern U.S. during April and May.

#### Female Activity and Condition versus Mating Status

In agreement with observations by Brower et al. (1977), females were generally in excellent condition through the overwintering period and across observation years. Leong et al. (1993) found little wing deterioration through the overwintering period in California. In contrast, Tuskes and Brower (1978) found the monarch wing condition deteriorated at California overwintering sites and suggested wing wear reflected mating activity. In support

of this hypothesis, James (1982a) and James (1984b) reported little change in wing condition in Australia in non-breeding transient and non-breeding overwintering monarchs, respectively. However, a large proportion of apparently old individuals occurred late in the season in a breeding overwintering population (James 1981). James also found that older monarchs were more likely to die at the breeding site. These findings support that mating or general activity increases wing wear as expected.

In Chapter 2, I found that early mating males (before 5 March) had wings in worse condition than those in the general overwintering population. However, they did not differ late in the season (after 4 March). Late mating males more closely resembled the general overwintering population; the latter changing little during the mating period. Females showed the opposite trend both early (before 5 March) and late (after 4 March) in the mating event.

My comparisons of both female age and activity versus her mating status showed 2 trends. Firstly, older females were more likely to have mated before the mass mating event. Secondly, a higher proportion of nectaring females had mated both previously and at the overwintering site as compared to clustered, flying, or drinking females. Furthermore, nectaring females had almost twice the percentage of individuals in poor condition as compared to all other activity categories (unpubl. data). These females may be expected to be in a weakened state of diapause (see above) since older females are more likely to invest in reproduction in late summer, and these would be expected to be in particular need of replenishing lipids.

Females seeking nutrients for egg production and remigration may profit simultaneously from obtaining nectar and nutritious spermatophores (Wells et al. 1993, Brower 1985, Zalucki 1993). Consistent with this idea, Brower et al. (1977) found that 63% of previously mated females collected before the mass mating event had very depleted lipid reserves. However, alternatively, nectaring females may be more likely to be mated because they encounter sexually active males at flower sources. Because nectaring males are also apparently older (Chapter 2, Alonso-M. 1996), they may be more likely to aggressively seek mating opportunities, as has been reported in summer breeding males (Pliske 1975, pers. obs.).

During the autumn migration transient aggregated populations occur in both eastern and western U.S. populations (et al. 1970, Brower 1995, pers. obs.) and in Australia (James 1993). At least in Australia and the southeastern U.S., these temporary aggregations are male-biased and matings do occur (Williams 1930, James 1982a, James 1984b, pers. obs.). These aggregations may be the main source of autumn matings before females reach overwintering areas.

Reproduction by older individuals during the southward migration supports the hypothesis put forth in Chapter 2 to account for why small, worn males predominate in mated pairs collected during the mass mating event in Mexico. Males that mate in late summer or along the southward migration are expected to 1) be older and/or in a lessened state of reproductive diapause, 2) have lower lipid reserves due to investment in mating, and 3) possibly experience more rapid senescence caused by juvenile hormone changes associated with reproductive activity (Herman 1985). These

costs may reduce their chances of successful remigration and lower competitive abilities at oviposition sites along the spring remigration routes. By mating at the overwintering site, these males avoid the costs of remigration, and mate location and possible male-male competition costs that occur thereafter, but they risk losing in sperm competition to males that successfully remigrate and mate with females at oviposition sites.

In Chapter 2 I found that, during the mass mating period, *in copula* females were in better condition than females collected from the general overwintering population. I hypothesized that this bias may result from a male choice for females in good condition. My observations during a semi-natural experiment (unpubl. data) support a male takedown preference for females in good condition. Also consistent with my hypothesis, Leong et al. (1995) found that a positive association between female size and the number of spermatophores in California overwintering females. However, in this study, using abdominal palpation, the occurrence of fresh spermatophores in females collected during the mass mating period was associated with females in poor wing condition. This is the same trend as was found in females that had mated before the mass mating event and is consistent with the hypothesis that females with higher nutrient needs seek nectar sources and, directly or indirectly, obtain spermatophores.

The discrepancy between my empirical finding that large, good condition females were found as mated pairs, while poor condition females collected from the general population were more likely to have fresh spermatophores, could be related to differences in

sampling methods. Ninety-six percent of all females collected during the mass mating event were in good to medium condition and these accounted for 93% of all freshly mated females. This is consistent with a male preference for good condition females. However, of the 4% of females in poor condition, more had mated than the population average. Because poor condition females represent such a small proportion of females available for mating they would not be highly represented in *in copula* pairs even if they were more likely to be mated as a group. This would be especially true for those that travel long distances to nectar sources, since they would not be represented in collections of mated pairs from within the colony boundaries. It is also likely that detection of spermatophores by palpation is relatively easier in females with low lipid and water contents.

Simultaneous male choice for good condition females and a higher propensity for poor condition females to seek out mates could account for my results. As suggested above, older females that mate during the southward migration may not be in reproductive diapause and may be more likely to seek nutrients provided by spermatophores (see Oberhauser 1989, Oberhauser 1992, Well et al. 1993). However, because multiple mating was extremely rare in all female phenotypes, polyandry as a mechanism to obtain nutrients appears limited at the Mexico overwintering site. Slow degradation of spermatophores may physically limit female ability to remate since successive spermatophores must fit into a single receptacle; the bursa copulatrix. Furthermore, successive matings in the absence of spermatophore degradation, may add excessive weight that must be carried during the long remigration (Oberhauser 1992). Such

wingloading constraints would not be expected to occur in summer breeders, and higher degrees of polyandry occur in those populations (see below). Alternatively, because these females seek nectar more often than the high quality females, they may be more likely to encounter males and therefore mate irrespective of nutrient needs.

#### Summary of Bursa Copulatrix Dissections and Palpation Results

Both palpation and bursa dissection (see Chapter 4 for details of these methods) gave results that were consistent with field observations of the timing and intensity the mass mating event. They both suggest that 1) mating occur during the southward migration and/or early on in the overwintering period, 2) the mass mating event is restricted to the last few weeks of overwintering, increases exponentially, and most matings may occur just before or just after the main exodus from the overwintering site, 3) female utilization of spermatophores may increase as the mating event begins, 4) many females leave the overwintering site without mating during the mass mating event, but not necessarily as virgins, and 5) multiple mating during the mass mating is rare, but already mated females may mate again during the mass mating period.

#### Frequency and Degree of Multiple Mating in Different Life Cycle Stages and in Different Populations

Table 5-4 compares the frequency and degree of multiple mating among Mexico and California overwintering females, spring



remigrants, summer breeders, and autumn migrants. A very high frequency and degree of polyandry is characteristic of summer breeders, late overwintering California females, and spring remigrants from the Gulf coast. The degree of polyandry at Australia overwintering sites has not been reported, but mating is nearly universal throughout the aggregation period. In contrast, late Mexico overwintering monarchs surpass only the northern U.S. autumn migrants that are known to be in reproductive diapause.

By the time autumn migrators reach the southern coastal U.S. they show levels of polyandry similar to those found in early overwintering females. Furthermore, the frequency and degree of polyandry does not increase during the mass mating period in Mexico. Together these findings suggest that most polyandrous matings observed at the Mexico overwintering site result from matings that occurred during the preceding autumn migration.

Mating in the late summer or during the autumn migration potentially reduces overwintering success. Depending on sperm viability, sperm precedence, and the timing and frequency of female remating, late autumn matings may influence the level of genetic panmixia of the following spring generation (Ehrlich and Ehrlich 1978, Tuskes and Brower 1978, Wells et al 1993).

The low overall frequency of multiple mating in late overwintering versus remigrating females suggests that matings obtained after leaving the overwintering sites are the primary genetic source of the first spring generation. This appears to be a major difference between eastern and western U.S. monarchs.

However, assuming sperm viability, autumn matings may contribute to the first spring generation.

Possible Proximate Explanations for Differences in Frequency and Degree of Mating in Mexico versus California Overwintering Females

Several non-exclusive proximate explanations could account for the differences between the frequency of mating and the degree of polyandry in Mexico and California overwintering monarchs. First, the difference could reflect sampling biases: In California extreme female mating frequencies occur only after almost all of the butterflies have dispersed from the site. Slightly earlier, lower estimates may be more representative and more consistent with the Mexico findings (e.g. Downes, in Williams et al. 1942, Hill et al. 1976, Leong et al. 1995). Second, California mass mating is more protracted resulting in higher frequencies of female mating. Thirdly, female-biased sex ratios should lessen male harassment in Mexico, while male-biased sex ratios should heighten male harassment in California. Finally, a more intense or longer female diapause in Mexico versus California overwintering monarchs could result in a stronger female resistance to mating attempts in Mexico. This differences could also lead to a higher male persistence in mating attempts in Mexico. These explanations are supported empirically and are interdependent.

Even with last male advantage in sperm precedence, males are expected to benefit from mating with virgin versus non-virgin females. Sperm competition avoidance could account for the

observed male rejection of previously mated females (Chapter 2). Such a male mating tactic could result in the limited level of polyandry observed in Mexico, but doesn't address the low levels of mating in general. Furthermore, the high degree of polyandry in late overwintering California females is apparently in conflict with this explanation. However, if most of these matings occurred in the previous autumn, sperm competition may be greatly reduced.

The intensity of sexual selection and costs and benefits of male mating tactics is also expected to differ in California and Mexico due to the dramatic difference in sex ratio, the length of the breeding period, and differing degrees of geographic separation between breeding and oviposition sites (Thornhill and Alcock 1983). For example, according to calculations by Wells and Wells (1992), California overwintering males are energetically able to mate up to 12 times. However, because Mexico overwintering males presumably remigrate at least initially on lipid reserves, they may well be much more constrained in spermatophore production and mate searching than California males (see Odendaal et al. 1985).

Female nutrient need has been suggested as a selective pressure leading to polyandry in monarchs (Brower 1985, Oberhauser 1989, Wells et al. 1993, Zalucki 1993). Following this line of reasoning we would expect that multiple mating should 1) increase female reproductive success, 2) be higher in Mexico than California, and higher in overwintering than summer breeders, due to expected differences in female nutrient needs, and 3) females should solicit multiple matings. However, these hypotheses have not been overwhelmingly supported.

Female nutrient need for successful remigration from Mexico is expected to be higher than in California. This reasoning is based on the following empirical data: 1) monarchs lose lipids throughout the overwintering period which is generally longer in Mexico, and 2) oviposition sites (and probably also nectar sources) are much farther from Mexico overwintering sites. However, the frequency and degree of polyandry is higher in California than Mexico; the opposite of the trend expected if females remate to gain nutrients.

If female nutrient needs control the level of polyandry, we would also expect female solicitation of mates (as suggested by Zalucki 1993) and a high rate of successful mating. Very long pre-copulatory interactions in Mexico (pers. obs.) and the apparently lower success rate of mating attempts at the Mexican overwintering site compared to summer breeders (pers. obs.) do not support female mate solicitation based on nutrient need. Multiple mating in Mexico could be counter-selected by the female's need to soar during the long-distance remigration due to an increased wing loading associated with mating (Oberhauser and Hampton 1995). The prediction that polyandry should be less common in summer breeding female compared to overwintering females is also not supported (Table 5-4). These arguments assume that nutrient and sperm supply needs are higher in the overwintering than in the summer breeders and are at least comparable in Mexico and California.

Females dispersal to avoid sexual harassment by males has been suggested to explain the male-biased sex ratios in late overwintering California monarchs. Female dispersal could lead to

lower levels of polyandry, but this cannot explain the observed differences between Mexico and California data, since females stay longer in Mexico where polyandry is lower. However, highly male-biased sex ratios in California could lead to stronger pressures for females to disperse from overwintering sites (see Shapiro 1970), and dispersal could constrain potentially higher levels of male-enforced polyandry. In summer breeders, females may bear the costs of male harassment (Clutton-Brock and Parker 1995) due to the benefits of oviposition sites. The costs and benefits of staying versus dispersing from California versus summer breeding sites may be tipped toward dispersal in California due to the absence of oviposition sites. This might account for the relatively higher mating frequencies in summer breeders.

Environmentally-Induced Variation in the Time of Female Mating  
During their Migratory Life History Cycle

Just-eclosed adults resulting from overlapping generations of geographically widely distributed late summer adults (Cockrell et al. 1993) will have experienced differing food plant conditions (species, quality, and quantity), daylength, temperatures, cloud cover, and cold front patterns. Goehring and Oberhauser (poster at Lepidopterists Society 46th Annual Meeting, 1995) reported that poor larval food plant quality was associated with reproductive diapause in emerging adults. Food plant quality may vary locally and even within plant patches.

Because post-eclosion temperatures are thought to be the primary determinant of the reproductive state of individuals (Barker

and Herman 1976, James 1983), unusually warm autumn temperatures could lead to the sometimes substantial levels of mating observed in pre-mass mating overwintering monarchs (James 1983, James 1984b, James 1986). Urquhart (1960) and Brower (1985) suggested that high temperatures sometimes experienced by autumn migratory monarchs could override diapause and could lead to continuously breeding founding populations in southern areas. Barker and Herman (1976) presented physiological evidence that this may occur.

These empirical findings may reflect and individually flexible oligopause as described for Australian migratory monarchs (James 1982b, 1993). Alternatively, southward migrating monarchs in the eastern U.S. may be a physiologically mixed group with some individuals in a true reproductive diapause and others in a non- or pre-diapause state. This scenario is more consistent with physiological work by Herman (1981) and others (see references in Herman 1993) that show an inability of individuals to immediately respond to favorable environmental conditions. A mixed population of true diapausing and non-diapausing individuals is also consistent with my findings that previously mated females were more likely to be older (see below) and that older, non-diapausing males were more likely to mate at a Mexico overwintering site (Chapter 3).

Either a population of environmentally responsive oligopausing monarchs or a mixed population of strict diapausing and non-diapausing individuals could account for the variable level of mating observed in pre-mass mating monarchs. While these 2 hypotheses are not mutually exclusive and not easily differentiated, the mixed

population hypothesis can account for both the inflexibility observed in physiological studies and the variability observed in field studies of populations.

Differences between the views of James and co-workers (e.g., James (1982b), James (1983), and James and Hales (1983)) and Herman and co-workers (see Herman (1985) (1993)) may primarily reflect the field versus laboratory nature of investigations, rather than evolved differences in diapause between Australia and North America. For example, field collections of migrators are likely to include individuals from a variety of locations that had been exposed to different environmental conditions. However, monarchs collected at a single location, perhaps on a single day are likely to have experienced very similar post-eclosion environments and would be expected to show little variation.

#### Environmentally-Dependent Costs and Benefits of Different Monarch Mating Tactics

Regardless of the mechanism, moderate to high levels of mating before the spring overwintering mass mating period have not been appreciated theoretically in terms of implications for overwintering mating strategies, remigration population demographics, and possible selection pressures for aggregated overwintering populations (Wells et al. 1993, and see below). Once an animal becomes reproductive the diapause condition is unlikely to be reinstated (Herman 1985, James 1993). Increased juvenile hormone levels associated reproductively active state speed senescence (Herman 1985) and increase lipid utilization (Barker and Herman 1976, Lessman and

Herman 1983). These findings suggest that reproduction during the autumn migration may carry a high price beyond the costs of mating by reducing overwintering success. Females may avoid some of these costs if they can mate without inducing gonad development, possibly through sustained flight (Herman 1985, 1993). Assuming that sperm remains viable, even with a last male advantage in sperm precedence, it appears that at least a portion of females remigrating northward the following spring lay eggs fertilized by males that they mated with during the southward migration. The benefits of autumn migration and early overwintering mating by males may also be substantial. It is therefore important to know when spermatophores were received as well as the fate of the sperm and nutrients transferred.

#### Summary of Comparisons of Mexico, California and Australia Overwintering Mating Ecology

Overwintering mating patterns observed at a single Mexican site over 4 years shows general similarities and some differences from the results of workers studying monarch mating behavior at overwintering sites in California and Australia. These are summarized in Table 5-5. In agreement with Herman (1993), differences appear to be a matter of degree and may reflect 1) within and between population genetic variability in the propensity to enter and remain in diapause, 2) a protraction of adult eclosion of in late summer overlapping generations, 3) differences in environmental conditions experienced in late summer, during movement and during the overwintering period, and 4) differences



in the costs and benefits of overwintering mating related to differences in the distance of overwintering areas from oviposition and food sources.

California mating ecology appears to be generally intermediate between Australia and Mexico sites. Colonies are male-biased in California and female biased in Mexico. Sex ratio is expected to both reflect and define mating system ecology (Emlen and Oring 1977). It is likely that good measures of sex ratio and unraveling the causes of sex ratio changes through time in California versus Mexico will contribute to our understanding of the differences in the degree and frequency of female mating.

My research suggests that variation in environmental conditions among populations, locations, phase of the life cycle, and years impose variation on monarch migration and reproductive physiology. These, in turn, determine the frequency, degree, and evolutionary and demographic significance of overwintering female mating.

Table 5-1. Comparison of sex ratio and percent mated females in 8 upper versus lower sub-colony samples matched by date, time of day and temperature 1985-1988. % Fresh = percent of palpated females with fresh spermatophores, % Mated = percent of females with fresh or old spermatophores.

Date	Sex Ratio						Percent Mated Females					
	Upper Colony			Lower Colony			Upper Colony			Lower Colony		
	n	% Male	%	n	% Male	%	n	% Fresh Mated	%	n	% Fresh Mated	%
4-8 Mar 86	106	57		19	63		20	30	45	7	43	71
20 Feb-6 Mar 86	570	33		114	47		50	16	42	20	25	30
11-12 Mar 86	109	17		113	48		25	28	56	25	44	76
15-17 Mar 86	87	22		60	60		30	37	63	24	67	83
17-19 Mar 86	651	20		284	26		45	28	58	25	48	76
28 Feb-2Mar 87	260	45		363	55		95	4	26	96	32	53
6-8 Mar 87	297	30		968	59		187	10	34	106	25	48
13-15Mar 88	553	43		694	39		61	16	33	40	18	28

Table 5-2. The total number of spermatophores found in the bursa copulatrix of monarch female butterflies overwintering in Mexico and in remigrants from the overwintering site collected in the spring of 1985 in the southeastern United States (see text).

	0	1	2	3	4	5	6	7	8	Unknown	Total (%Mated)
<u>Overwintering</u>											
Number	451	172	21	0	1	0	0	0	0	24	669
Percent in group	67	26	3	0	0.1	0	0	0	0	4	33
<u>Spring Remigrants</u>											
Number	0	5	11	10	7	6	5	3	1	0	48
Percent in group	0	10	23	21	15	13	10	6	2	0	100

Table 5.3. Tukey-type multiple comparison testing for differences in proportion of females with old spermatophores (1985) and fresh spermatophores (1986) among the four activity categories: clustered, drinking, flying, nectaring (see text).

Year	Comparison	q	q .05,4	Conclusion
1985	Flying vs Drinking	5.72	3.63	Reject Ho
	Flying vs Clustered	3.59	3.63	Accept Ho
1986	Nectaring vs Drinking	8.61	3.63	Reject Ho
	Nectaring vs Clustered	8.42	3.63	Reject Ho
	Nectaring vs Flying	6.32	3.63	Reject Ho
	Flying vs Drinking	2.46	3.63	Accept Ho

Summary of Group Differences				
1985:	Drinking	Clustered	Nectaring	Flying
Old/Total (% Old)	(91/268) (34)	(116/282) (41)	(55/120) (46)	(101/191) (53)
1986:	Drinking	Clustered	Flying	Nectaring
Fresh/Total (% Fresh)	(16/71) (18)	(63/205) (24)	(25/70) (26)	(39/52) (43)

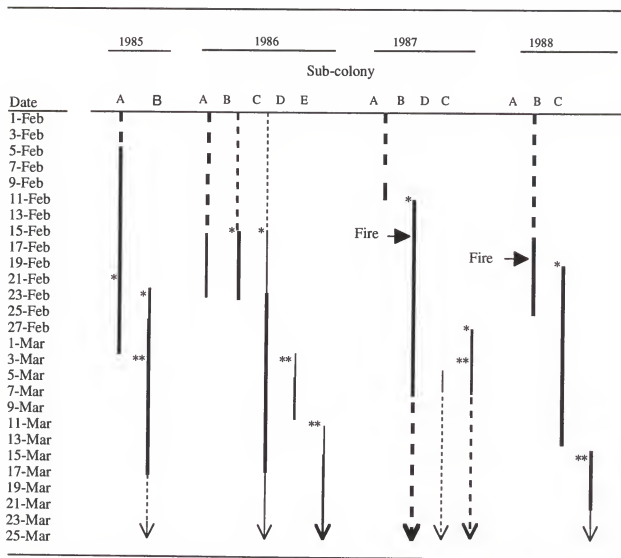
Table 5-4. Comparison of multiple mating at different times in the life cycle and among eastern US, western US and Australian monarch populations. Reference codes: a = Brower et al. 1977, b = Brower 1985, c = this paper, d = Downes, in Williams et al. 1942, e = Leong et al. 1995 (\* combined > or = 3 spermatophores), f = Hill et al. 1976, g = Pliske 1973, h = Suzuki and Zalucki 1986.

PERCENT IN GROUP														
Number of Spermatophores														
Sample	0	1	2	3	4	5	6	7	8 to 10	Unkn.	(N)	% Mult. Ref.		
												% Mated	Mated	
OVERWINTERING														
<u>Mexico</u>														
Jan 85	85	11	2	2	0	0	0	0	0	.	111	15	27	a
Jan 77	17	5	0	0	0	0	0	0	0	.	99	22	23	b
Jan-Feb 49	38	6	0	<1	0	0	0	0	0	6	234	51	25	c
Feb 303	78	20	2	0	0	0	0	0	0	0	303	22	10	b
Feb-Mar 54	39	3	0	0	0	0	0	0	0	4	214	46	16	c
Mar-Apr 42	42	13	0	0	0	0	0	0	0	.	251	55	23	b
<u>California</u>														
Oct 63	33	0	0	0	0	0	0	0	0	.	12	33	0	d
Oct 49	33	18	0	0	0	0	0	0	0	.	45	51	34	e
Nov 59	28	11	1	.	.	.	.	.	.	.	140	41	62	e
Dec 53	34	8	5	.	.	.	.	.	.	.	129	47	28	e
Dec 63	29	6	2	0	0	0	0	0	0	0	100	29	22	b
Jan 48	31	15	6	.	.	.	.	.	.	.	134	52	40	e
Feb 30	32	29	9	.	.	.	.	.	.	.	142	70	54	e
Feb 25	44	22	7	0	2	0	0	0	0	0	100	75	41	b
Feb 0	16	48	20	4	4	4	4	4	0	0	25	100	84	f
32	63	5	0	0	0	0	0	0	0	0	19	68	8	d
Mar 5	14	24	57	.	.	.	.	.	.	.	138	95	85	e
April 0	0	40	0	20	40	0	0	0	0	.	5	100	100	d
<u>Southern Florida (Continuous Breeders)</u>														
Dec 12	6	18	29	24	6	0	6	0	0	.	17	88	93	b
SPRING REMIGRANTS														
<u>Southeastern US</u>														
Mar-Apr 0	10	23	21	15	13	10	6	2	.	.	48	100	90	c
SUMMER BREEDERS														
<u>Trinidad</u>														
July-Aug 4	46	18	13	7	9	2	0	1	.	.	91	96	52	g
<u>Massachusetts</u>														
July-Aug 2	20	20	10	20	7	10	2	10	.	.	41	98	80	b
<u>Australia</u>														
Jan (sum) 2	5	11	12	15	18	10	10	15	.	.	97	98	95	h
AUTUMN MIGRANTS														
<u>Massachusetts</u>														
Sept 96	4	0	0	0	0	0	0	0	0	.	56	4	0	b
<u>Coastal Florida</u>														
Oct 71	21	4	2	2	0	0	0	0	0	.	52	29	27	b

Table 5-5. Comparison of overwintering colony and mating ecology among Mexico, California, and Australian overwintering monarchs (see text for references).

Comparison	Mexico	California	Australia
Migration Distance	Long	Medium	Short
Availability of Nectar/Host Plants	Very Little	Little	Little-Common
Overwintering Environment	Less Mild	Mild	Mild
Colony Size	Large	Medium	Small
No. of Colonies/Popul. Size	Few	Many	Many
Occurrence of Transient Colonies	Yes	Yes	Yes
Reproductively Active OW Colonies	No	Yes	Yes
Length of Overwintering Period	Long	Intermediate	Short
Length of Diapause	Long	Intermediate	Oligopause
Length of Mass Mating Period	Very Brief	Variable	Throughout
Ending Sex Ratio Bias	Female	Male	Male
Wing Wear	Little	Moderate	Little-Moderate
Pre-aggregation Mating	Variable	Variable	Common
Mass Mating	Yes	Yes	Absent
Females Mated at End of Mass Mating	< 40%	Near 100%	100%
Degree of Polyandry	Very Low	Low-Medium	?
Late Season Lipid Levels	Low	Intermediate	Low
Environ. Cues for Mating/Dispersal	Yes	Yes	No?

Figure 5-1. The approximate date of formation, departure, relative size, and relative uphill-downhill position of sub-colonies observed in Sierra Chincua, Mexico during 4 overwintering seasons (1985-1988). The overwintering colonies became increasingly dynamic through time in all years as they moved downhill. Only in 1987 did a sub-colony form uphill from an existing colony (notice colony D in 1987 formed uphill to the pre-existing colony C; see text for explanation). The approximate dates of when mating was first observed and when mating came into full swing are indicated by a single or a double asterisk, respectively. In 1988 mass mating was apparently deferred due to the unusually cold weather late in the season. Dramatic increases of mating activity were consistently associated with downhill movement and fission of the overwintering colony into distinct sub-colonies (A to B, etc.). Mating was also consistently more common in the downhill than the uphill sub-colonies when they co-occurred.





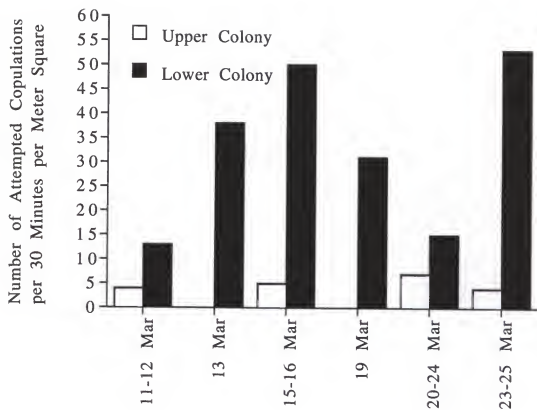


Figure 5-2. Rates of attempted copulations in 6 matched samples of upper and lower sub-colonies: 1986.

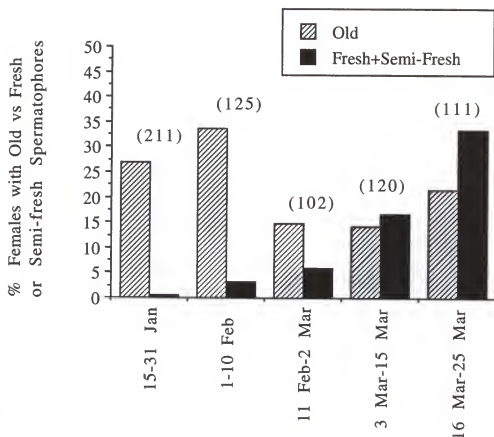


Figure 5-3. Changes in female mating status through time during the 1985 overwintering season based on bursa copulatrix dissections (n = 669).

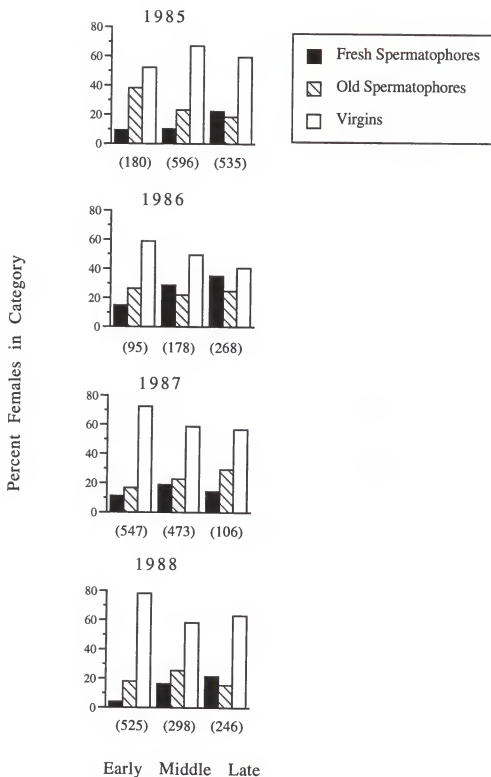
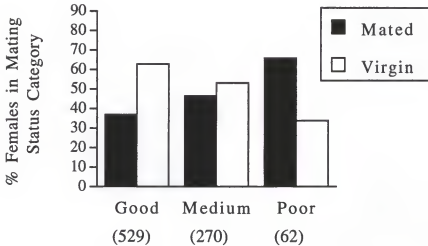


Figure 5-4. Female mating status through time as assessed by palpation. Early = 15-29 February, Middle = 1-12 March, Late = 13-29 March.

## A. Before Mass Mating (2 - 19 February)



## B. During Mass Mating (20 February - 25 March)

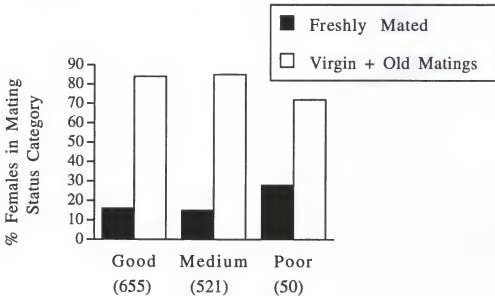


Figure 5-5. Percent females in mating status category for good, medium and poor wing condition categories: 1985.

A. Before mass mating. B. During mass mating.

CHAPTER 6  
POLYANDRY AS A SELECTIVE AGENT IN THE EVOLUTION OF  
MONARCH OVERWINTERING AGGREGATIONS

Introduction

In the autumn, monarchs that occur west of the Rocky mountains migrate to scores of overwintering sites along the Pacific coast of California (but see Wenner and Harris 1993), while those east of the Rocky mountains migrate to a few isolated colonies in the Transverse Neovolcanic Belt in central Mexico (Brower 1985, Brower 1995). Overwintering sites are extremely small and restricted to tiny areas within islands of highland relic Oyamel fir forests, *Abies religiosa* (H. B. K. Schl. and Cham. (Pinaceae) (see references in Brower 1995). Mexico overwintering areas range from 0.1 to 5 ha, and are characterized by their extreme butterfly densities reported to be approximately 10 million per ha (Brower et al. 1977, Calvert in prep., in Brower 1985). These are 2 orders of magnitude larger than California overwintering colonies (Brower 1985) which range from 40,000 to over 200,000 individuals (Hill et al. 1976, Tuskes and Brower 1978). Still smaller winter aggregations occur in Australia (Smithers 1965, James 1984b, James 1993). However, extreme population densities are also characteristic of these smaller overwintering sites.

Several non-exclusive hypotheses to account for the extreme adult densities at overwintering sites have been put forth and supported empirically. These fall into 2 general categories. First, moderation of seasonal and diurnal abiotic conditions (Calvert et al. 1982, Chaplin and Wells 1982, Masters et al. 1988, Wells et al. 1990, Anderson and Brower 1993, Leong et al. 1993, Anderson and Brower 1995) is thought to enhance conservation of lipids (Tuskes and Brower 1978, Chaplin and Wells 1982, Masters et al. 1988, Wells et al. 1990), reduce vulnerability to freezing (Anderson and Brower 1993, Anderson and Brower 1995), and maintain reproductive diapause (Brower et. al. 1977, Tuskes and Brower 1978, Urquhart and Urquhart 1977, Brower 1988). Second, aggregated overwintering colonies are thought to minimize potential predation (Tuskes and Brower 1978, Brower and Calvert 1985, Calvert et al. 1979). Wells et al. (1990) and Wells et al. (1993) acknowledged these benefits of aggregation, but maintained that they do not adequately account for the evolutionary origin of dense overwintering populations. They proposed that spermatophore-derived nutrients obtained by females during the spring 'frenzied' mating activity is an important selective agent leading to the extreme overwintering population densities.

Reproductive advantages and population effects of the mass mating event, including prevention of race formation and maintenance of the generalized migratory behavior, have been previously proposed (Tuskes and Brower 1978, Herman 1981, Brower 1985). However, Wells et al. (1990) and Wells et al. (1993) rightly point out that the importance of the mass mating event has

not received appropriate attention as an important selective agent in monarch overwintering behavior.

Monarch overwintering aggregations bring the adults of broadly distributed late summer generations into close proximity for several months. Just before the northward remigration in spring, the hormonally controlled reproductive diapause is broken (Herman et al. 1989) and a mass mating activity ensues. From Mexico females must remigrate, at least as far as the southern U.S., to milkweed patches where they lay eggs (Van Hook and Zalucki 1991, Malcolm et al. 1993, Malcolm, Van Hook, Brower unpubl. data). California overwintering females travel less far, but cover considerable remigration distances (Nagano et al. 1993, Wells et al. 1993, but see Wenner and Harris 1993, for review see Brower 1995).

Environmentally controlled migration, overwintering reproductive diapause, and remigration coordinate monarch life history so that they can successfully utilize seasonally abundant larval milkweed resources. However, this complex life history strategy hinges on successful migration to, and remigration from, overwintering sites that are far removed from larval oviposition sites, survival during the several month long overwintering period, mate acquisition, and female remigration. The importance of maintaining adequate energy supplies for fueling these activities is therefore thought to be paramount to monarch biology (Brown and Chippendale 1974, Tuskes and Brower 1978, Chaplin and Wells 1982, Ackery and Vane-Wright 1984, Brower 1985, Masters 1993, Wells et al. 1993).

Successful female remigration is a particularly critical link in the monarch's annual life cycle. Lipids are depleted throughout the overwintering period (James 1984a, Brower 1985, Masters et al. 1988, Wells et al. 1993), while nutrients from nectar sources are scarce at overwintering sites (Brower et al. 1977, Tuskes and Brower 1978, Brower 1985, Masters et al. 1988). Because spermatophores contain both sperm and nutrients, the timing, frequency, and quality of female mating before, during, and after overwintering influence re-colonization potential and the first spring generation population demographics (Ehrlich and Ehrlich 1978, Wells et al. 1993). Several authors (Herman 1981, Brower 1985, Suzuki and Zalucki 1986, Oberhauser 1989, Wells et al. 1993, Zalucki 1993, Chapter 3) have suggested that male-derived nutrients obtained from spermatophores may thereby contribute significantly to female remigration potential and reproductive success.

Using a theoretical population model, Wells et al. (1993) predicted that increased female longevity and higher fecundity resulting from increased lipid reserves enhance long-term persistence of overwintering monarch populations under adverse conditions. Wells et al. (1990) further hypothesize that extreme overwintering population densities are selected through reproductive benefits associated with widespread multiple mating. These hypotheses are based on empirical evidence that 1) the mass mating event is characteristic of aggregated overwintering monarchs (Hill et al. 1976, Urquhart 1976, Urquhart and Urquhart 1976, Brower et al. 1977, Chapter 3, but see James 1984b, and James 1993), but is absent in summer breeding populations (Wells et al. 1993), 2) female



mating is "universal and multiple" by the end of the overwintering period at California overwintering sites (Hill et al. 1976), and 3) California overwintering females show decreasing levels of lipids until the mass mating period begins when lipids increase, while males continue to lose lipids (but see Tuskes and Brower 1978).

Following the arguments of Wells et al. (1990) and Wells et al. (1993), I tested whether the quantity of lipids transferred per mating, together with the frequency and degree of observed polyandry (Chapter 5), can account for the level of lipid content increase found in late overwintering females in California. Cardenolides obtained from larval food plants are also transferred in spermatophores (Brower 1985). These compounds are known to function in adult chemical defense and may protect eggs. I therefore also considered whether the quantity and quality of cardenolides obtained by multiple mating could significantly boost female defensive chemicals.

### Methods

The methods of collection and determination of the frequency of female mating in the overwintering population and the degree of multiple mating are reported in Chapters 4 and 5.

For lipid and cardenolide analyses I dissected the bursae of 42 females collected from *in copula* pairs on 9 and 17 March 1985. Upon de-coupling the pair, wet weight (to the nearest 0.1mg), right forewing length (to the nearest 0.5 mm), and wing condition were recorded for both sexes. Wing condition was used as an indicator of

age and was classified as good, medium or poor following the methods described in Chapter 2. Butterflies were stored in glassine envelopes on ice in the field until frozen in a conventional freezer within 4 to 5 days. Frozen females were thawed, re-weighed, and their bursa dissected as described in Chapter 4. Fresh spermatophores were removed and weighed to the nearest 0.1 mg on a Mettler AK 160 balance. From these I selected 30 female-male pairs, based on the presence of an intact fresh spermatophore in the bursa, to analyze the lipid and cardenolide content of the males, females, and the just-transferred spermatophores. I assume the fresh spermatophores present in the bursa copulatrix were passed by the male with which the female was mating when captured.

Individual butterflies and spermatophores were dried at 60 ° C for 16 h and then individually extracted for lipids in petroleum ether (Walford 1980, May 1992). Following the spectrophotometry methods of Brower et al. (1982) and Malcolm et al. (1989), cardenolide concentrations were measured at a wavelength absorbency of 622 nm using a Perkin Elmer UV/VIS Spectrometer Lambda 2 S spectrophotometer. Butterflies were analyzed for cardenolide content individually but, due to their small size, spermatophores were analyzed in 6 pooled samples of combined lean material from 5 spermatophores each.

## Results

Table 6-1 gives the wet weight, dry weight, percent lipids (of total dry weight), and cardenolide concentration of 30 mated pairs

and their just-transferred spermatophores. Mated pairs were low in lipids. The means were 42 mg and 32 mg, for females and males, respectively. This compares to a mean of 59 mg for combined sexes reported by Brower (1985) for late Mexican overwintering monarchs.

The average wet weight of 30 just-transferred spermatophores was 16.4 mg and represented a male investment of approximately 3.8% of his total body weight. The average spermatophore dry weight was 5.2 mg. Spermatophores contained approximately 68% water. Spermatophore wet and dry weights were less than 4% of female wet and dry weights (see Table 6-1).

Male dry weight was significantly positively correlated with spermatophore dry weight (Spearman rank-order correlation coefficient (corrected for ties);  $r_s = 0.46$ ,  $Z = 2.48$ ,  $p = 0.01$ ,  $n = 30$ ). However, neither male dry weight or spermatophore dry weight were correlated with the weight of the lipids present in the spermatophores ( $r_s = 0.21$ ,  $Z = 1.14$ ,  $p = 0.25$ ,  $n = 30$ ;  $r_s = 0.15$ ,  $Z = 0.81$ ,  $p = 0.42$ ,  $n = 30$ , respectively). Neither spermatophore dry weight nor spermatophore fat weight varied between good versus combined medium and poor male wing condition categories (Mann-Whitney U;  $Z$  (corrected for ties) = -0.93,  $p = 0.35$ ,  $n = 30$ ;  $Z$  (corrected for ties) = -1.2,  $p = 0.23$ ,  $n = 30$ , respectively).

Approximately 15% of the fresh spermatophore dry weight was lipid. This was comparable to that found in the mated butterflies (21% for females and 16% of males; see Table 6-1). The average spermatophore lipid weight was 0.7 mg. This represented approximately 1.7% of total female lipid weight (0.7 mg/42 mg) and 2.2% of total male lipid weight (0.7 mg/32 mg). Female lipid weight

did not include the lipids from the received spermatophore since the spermatophores had been removed.

Using pooled spermatophore samples, the estimated cardenolide content of a fresh spermatophores was approximately  $1 \text{ ug} \pm 0.3 \text{ SE}$  (range 0-2.1). This compares with  $72 \text{ ug} \pm 14 \text{ SE}$  (range 0-253) and  $95 \text{ ug} \pm 20 \text{ SE}$  (range 0-501) for the males and the females from which the spermatophores were transferred. Fresh spermatophores represent only about 1% of the average cardenolide content of the adults. This is because spermatophore concentration was only about half that of the adults and because the fresh spermatophore weighs only a small fraction of the adult's body weight (see Table 6-1).

### Discussion

#### Multiple Mating as a Primary Selective Pressure for Monarch Overwintering Aggregations

Wells et al. (1990) and Wells et al. (1993) suggest that female monarch nutrient need for remigration from overwintering sites have been a major selective force in shaping the tight aggregations patterns of overwintering colonies. High density colonies maximize multiple mating through which females obtain fuels necessary for successful remigration. Wells et al. (1993), using a theoretical population model, showed that increased lipid resources available to remigrating females enhance long-term persistence of overwintering monarch populations under adverse conditions. Wells et al. (1990) then used a physical-kinetics model to show that the percentage of

female mating by females and the degree of polyandry are maximized at predicted aggregated population sizes close to those observed in California.

Their hypothesis assumes that 1) multiple spermatophores found in late overwintering females were obtaining during the aggregated mass mating period, 2) lipids received through multiple mating are a limiting factor in female remigration and reproduction, and 3) female multiple mating is advantageous to both males and females. They did not measure the actual frequency of multiple mating, the degree of polyandry, nor the amount of lipids transferred through spermatophores.

If gaining lipids through multiple mating is a primary adaptive function of the spatial and temporal character of the overwintering colony, 1) mating should be universal or nearly universal in overwintering females, 2) the frequency and degree of multiple mating should be high, 3) observed levels of polyandry should account for increased levels of lipids observed in late overwintering females, and 4) the degree of multiple mating should be positively associated with the degree of female nutrient needs.

My empirical data for Mexico do not support the hypothesis that female nutrient needs are shaping overwintering colony structure put forth by Wells et al. (1990) and Wells et al. (1993) . The potential for females to gain lipids or cardenolides through multiple mating appears to be limited at Mexican overwintering sites due to the limited frequency of female mating during the mass mating event, the rarity of multiple mating during the mass mating

event, and the small quantities of materials transferred in spermatophores.

### Frequency and Degree of Mexico Overwintering Female Mating

I discuss the rarity of mating and polyandry observed during the mass mating in Chapter 5. Here, I briefly summarize those results. Female mating frequency increased exponentially throughout the approximately 6 week long mass mating period. Approximately 45% of female matings occurred during the final week before remigration.

The frequency of female mating during the mass mating event is much lower in Mexico than California overwintering females. Only 20% of all Mexico overwintering females collected during the mass mating period had recently mated in 1985. Hill et al. (1976), Tuskes and Brower (1978), Herman et al. (1989), Leong et al. (1995) all report near universal mating in California late overwintering females. Interestingly, in Australia, where mass mating does not occur, most overwintering females are mated (James 1993). There mating occurs soon after colony formation and virgins never account for more than 30% of the females.

Apparently, between 65 and 86% of females disperse from the Mexican overwintering site without mating during the mass mating period. Many dispersing females may not be virgins, however, due to the commonness of mating before the mass mating period. Mating is also common along the spring remigration route. By the time remigrating females reach the southern U.S. 100% are mated.

Multiple mating was rare in Mexico. Only 7% of all dissected females, or 21% of mated females were multiply mated. Leong et al. (1995) found 33% of all females, and 54% of mated females were multiply mated at California overwintering sites. Including those mated an unknown number of times, only 11% of mated females were mated more than twice in Mexico.

While the proportion of females with fresh spermatophores increased during the mass mating event in Mexico, there was no associated increase in the frequency of polyandry from that measured before mating began. These data do not support the prediction by Wells et al. (1993) that the number of spermatophores per female should increase as the frequency of matings increase due to the highly aggregated reproductive population.

Female nutrient needs may be important in determining female mating patterns. I found a higher percentage of non-virgins both before and during the mass mating in old versus young females. Nectaring females were also more likely to be mated. Such females may seek out copulations (Zalucki 1993). However, female needs cannot be separated from male imposed matings, since nutrient deprived females are more likely to visit flowers where males takedown females. In fact, mating could be viewed as a cost to receive nectar in these females. Regardless of female nutrient needs, and in contrast to suggestions by previous workers, (e.g. Herman 1981, Brower 1985, Wells et al. 1993), the overall low frequency of mating and degree of multiple mating at the Mexico overwintering site offers negligible refueling resources for remigrating females.

### Male Investment in Spermatophores

Using spermatophore weights as an estimate, in agreement with Oberhauser (1988, 1992), and Forsberg and Wiklund (1989), males do invest heavily in spermatophores. The size of male investments in spermatophores varied greatly, but on average wet weights of spermatophores (16.4 mg) were similar to those found in wild caught summer breeding males (Oberhauser 1992) and represented approximately 4% of the male's total body weight. Using percent body weight as an estimate of male investment in spermatophores, overwintering males invest similarly as summer breeders. Virgin male summer breeders invested 5 - 10% of their body weight (Oberhauser 1988), and laboratory reared males allowed 3 days between matings invested 7.6% of their body weight (Svärd and Wiklund 1988a). Spermatophore wet and dry weights were approximately 4% that of females. Based on weight, multiple mating could offer substantial material benefits to females.

Male dry weight was positively correlated with spermatophore dry weight as has been reported for monarchs by Oberhauser (1988). However, neither male dry weight or spermatophore dry weight were correlated with spermatophore lipid weight. If lipids are the limiting nutrient that females obtain from spermatophores, this lack of correlation supports the suggestion of Marshall and McNeil (1989) that spermatophore size may not be a good indicator of male investment. However, Oberhauser (1992) did find a general positive association between spermatophore dry weight and nitrogen weight in all but very recently mated male monarchs.



Potential for Spermatophores to Account for Lipid Weight  
Increases Observed in California Late Overwintering Females

My data suggest that lipid levels obtained through polyandrous matings cannot account for the increased lipid levels in late overwintering females observed by Wells et al. (1993). Lipid weight of mated pairs averaged 32 to 42 mg, falling within the range of Mexico (Brower 1985, Alonso-M. 1996) and California (Tuskes and Brower 1978) late overwintering monarchs and reproductively active monarchs in Australia (James 1984a). The mean lipid weight, expressed as percent lean dry weight (to compare with Wells et al. 1993), was lower in Mexico than reported for late overwintering California monarchs (27 versus 41% for females and 21 versus 29% for males, Mexico versus California respectively, see Wells et al. (1993)), but showed the same trend of relatively higher percent lipids in females.

Since both sexes lose lipids throughout the overwintering period (Tuskes and Brower 1978, Chaplin and Wells 1982, James 1984a, Brower 1985, Masters et al. 1988, Alonso-M. 1996), females apparently either start with higher levels than males, or are better at lipid conservation than males (see Tuskes and Brower 1978, Alonso-M. 1996), and/or boost lipid resources through feeding or multiple mating. If increased opportunity to obtain lipids was a primary adaptive advantage of the dense aggregation in overwintering colonies, we would expect higher levels of mating and lipids in Mexican late overwintering females than California overwintering females. This is based on the assumptions that Mexico monarchs

must travel much farther to reach oviposition sites (see Wenner and Harris 1993, Brower 1995). In agreement with Brower (1985) the low lipid levels observed in both sexes in my study suggest that late overwintering females are constrained in remigration potential and likely must feed along the remigration routes northward.

Spermatophores do contain lipids in similar concentrations as observed in the mated pairs from which they were obtained, but due to their relatively small size and the limited degree of polyandry observed, they appear not to be able to account for the boost in lipids observed by Wells et al. (1993) in California overwintering females. Spermatophore-derived lipid weight accounted for approximately 2% of both male and female lipid weight. Using the linear rate of lipid loss observed in California overwintering monarchs before the mass mating event, females were expected to contain 20% lipid weight (of lean body weight), but they were observed to have 40.5% during the mating event (see Wells et al. 1993, Figure 1, p. 62). Assuming that California overwintering female average lean weight was 159 mg (see Brower 1985 for Mexican overwintering females), females would have to gain approximately 32 mg of lipids to account for the lipid increase during the mass mating event observed in females, but not males. If California overwintering males contribute similar levels of lipids per spermatophore as observed in my study, this indicates that females would have to mate approximately 46 times to account for the increase in lipids that Wells et al. (1993) attributed to polyandry.

The maximum degree of polyandry reported in California overwintering females is 7 (Hill et al. 1976) and the maximum

observed in any population, to my knowledge, is 14 in remigrating monarchs (pers. obs.). Assuming 1) most mass mating is constrained to the last 2 weeks of the mating period (Hill et al. 1976, Leong et al. 1995), 2) males can mate only once per day (Wells and Wells 1992), and 3) all males mate randomly with all females (not likely), we would expect no more than 14 matings per female. Furthermore, observations during overwintering colony formation by Downes (in Williams et al. 1942), Tuskes and Brower (1978), Herman et al. (1989) and Leong et al. (1995), suggest that at least a portion of the total number of spermatophores in late overwintering females were obtained before the aggregation period, and so cannot act as a selective pressure for aggregation.

Although lipids obtained through multiple matings cannot account for the observed levels of overwintering female lipids associated with the mass mating event, the average lipid contribution per spermatophore of 0.7 mg may be a significant boost for overwintering survival, remigration and reproduction. However, speculation suggests that this avenue is limited for females. For example, Masters et al. (1988) calculated that monarchs burn approximately 0.6 mg of lipid per day while resting and much higher rates ranging from approximately 4 to 11.5 mg when engaged in various activities. This suggests that even multiply mated females cannot significantly increase their lipid levels. According to estimates by Brower (1985), females can travel about 5.5 km per mg of lipid using powered flight, and perhaps much farther if they soar and use winds during the remigration as suggested by Gibo (1981). Females that mate 7 times could theoretically go an extra 27 km.

Considering the overall remigratory route, the potential costs of mating and flower resources thought to be available during remigration, it seems very unlikely that potential to refuel for remigration is a primary selective force for polyandry. Lipids are also used for yolk production (Pan and Wyatt 1976), and multiply mated females lay more eggs (Oberhauser 1989). The value of small additions in lipid weight for egg production may be appreciable. However, considering the relatively small quantities of lipids available through multiple mating, the low levels of lipids observed at the end of the overwintering period, and the costs of mating (see Hill et al. 1976, Pliske 1975, Suzuki and Zalucki 1986, Oberhauser 1989, Chapter 3) it appears that spermatophores must be a very limited source of lipids.

Spermatophores may provide other nutrients that are limiting to females (Oberhauser 1989, Oberhauser 1992, Oberhauser and Hampton 1995, but see Svärd and Wiklund 1988a). However, assuming nutrient needs are higher in late overwintering females than summer generation females, my results suggest that female nutrients needs do not account for high levels of polyandry. Furthermore, nutrient need cannot account for the tight aggregations found in Mexican overwintering sites since polyandry is rare at those sites (Chapter 5).

Although Wells et al. (1993) dismiss nectar as a significant source of lipids because males did not show a similar increase in lipids at the end of the overwintering period, they did not rule out a sex difference in nectaring behavior. Since males generally stay at California overwintering sites while females remigrate, we might

expect such a difference (see Odendaal et al. 1985). Furthermore, the trend in sex differences in lipid content observed by Wells et al. (1993) may have been anomalous. Tuskes and Brower (1978) found that, although females started out with a higher percent lipid content, they lost more through the overwintering period than males. Although, Leong et al. (1995) found that the average female wet weight increased at the end of the overwintering period at a California site, the increase reflected a difference in female body size in early versus late season samples rather than a difference in degree of polyandry.

#### Potential for Spermatophores to Contribute Significant Cardenolide Quantities to Late Overwintering Females

Both male and female average cardenolide contents (72 and 95 ug, respectively) of mated pairs were within the values most commonly found by Fink and Brower (1981) from Mexican overwintering monarchs. In agreement with Achey (unpubl. data, in Brower 1985), I found that fresh spermatophores do contain cardenolides. Spermatophore cardenolide concentration was about half of that found in the adults. However, due to the lower concentration, and primarily due to the small mass of spermatophores, they represent only about 1% of the average cardenolide content of the males and females from which they were transferred.

According to Fink and Brower (1981), because Mexican overwintering monarch contain almost exclusively weakly emetic cardenolides associated with the larval food plant *Asclepias syriaca*,

total amounts per butterfly must be at least 323 ug to cause emesis in the average avian predator. Since each spermatophore provides on average 1 ug of cardenolide, in order to boost the average mated female's cardenolide content from that observed to one emetic dose, a female would have to mate an estimated 228 times ( $323 \text{ ug} - 95 \text{ ug} = 228 \text{ ug}$ ,  $228 \text{ ug} / 1 \text{ ug per spermatophore} = 228$ ). Thus, the quantity of cardenolides transferred to females by males during mating, even in multiply mated females, probably does not significantly contribute to the unpalatability of females. Much smaller amounts may be necessary to instill protection to eggs since they are so small. However, the small amount available per mating and the relatively low levels of polyandry observed suggest that attainment of cardenolides is not a driving pressure of multiple mating in monarchs overwintering in Mexico.

California overwintering monarchs and summer breeders show variable cardenolide levels and utilize plants with much higher emetic potential (Brower and Moffit 1974, Fink and Brower 1981). Multiple mating is more common in both these areas than in Mexico overwintering monarchs. It may be possible that spermatophores could contribute to female or egg protection in these populations. However, the low concentrations and small size of spermatophores suggest their contributions to emesis is likely negligible.

#### Other Evidence Against Polyandry as a Selective Agent in the Evolution of Monarch Overwintering

If increased opportunity for obtaining nutrients through multiple mating is a primary driving force selecting for the tightly

aggregated overwintering colonies as suggested by Wells et al. (1993), several predictions follow. First, mating frequencies should be lower in summer breeders because females do not need to travel to larval food sources and because nectar sources are readily available. Second, aggregation size should not increase beyond the size and density necessary to maximum female polyandry. Third, both males and females should benefit from polyandry. Fourth, the frequency of mating and the degree of multiple mating should be high at both Mexico and California overwintering sites. Finally, multiple mating at overwintering sites should be high before the number and density of individuals decreases as spring remigration begins. These predictions are inconsistent with empirical results from a variety of other workers. I review these below.

Wells et al. (1993) argue that monarchs neither tightly aggregate or show mass mating in summer populations (Etchegaray and Nishida 1975, Smithers 1977, in Well and Wells 1992) because food is more abundant and energy transfer is not as important for female survival as in overwintering females. However, mating is nearly universal in summer breeding females in the absence of mass mating and the degree of polyandry is apparently higher than at overwintering sites (see Table 5-4, Chapter 5; Pliske 1973, Brower 1985, Suzuki and Zalucki 1986, Zalucki and Suzuki 1986, Oberhauser 1988, Zalucki 1990, pers. obs.). In Australia, aggregated overwintering monarchs do not show a mass mating event, but most females are mated (James 1993). The presumed association between aggregation and mass mating and between mass mating and multiple mating is therefore questionable.

Mexican overwintering sites are several orders of magnitude larger than California overwintering sites (Tuskes and Brower 1978, Brower 1985). The hypothesis of Wells et al. (1990) cannot account for this difference. Their model shows that after overwintering sites reach between 50,000 and 200,000 further increases in numbers do not further increase opportunity of widespread polyandry. Calvert et al. (1979) suggests that anti-predation benefits from large, aggregated populations continue to provide benefits as the population continues to increase.

The hypothesis put forth by Wells et al. (1990) and Wells et al. (1993) assumes that both males and females benefit from maximized female polyandry. Instead male and female reproductive interests are likely to be in conflict (Oberhauser 1989, 1992, Chapter 2). Due to last male sperm precedence, males should be selected to both prevent or delay female remating and to remate with non-virgin females. In agreement with empirical findings of low levels of polyandry in Mexico, these male tactics may actually reduce the degree of multiple mating in very dense populations compared to dispersed, continuous breeders. However, in dense population aggregations, extremely male-biased sex ratios should increase the degree of polyandry, while extremely female-biased sex ratios should decrease the degree of polyandry (Thornhill and Alcock 1983). Unlike, the hypothesis put forth by Wells et al. (1993), this scenario 1) is consistent with the low degree of polyandry in Mexico and the higher degree of polyandry in California, and 2) does not assume the unlikely lack of conflict of interest between the sexes in the timing and frequency of female mating.



The low level of mating observed in Mexico as compared to California overwintering females is also inconsistent with the nutrient maximization hypothesis. The low levels of mating observed in Mexico (Chapter 5) suggest that females either are not limited by sperm or spermatophore-derived nutrients, or the cost of multiple mating is especially high at Mexico overwintering sites. In support of the second alternative, Oberhauser and Hampton (1995) suggested that wing loading constraints imposed by the extra weight of multiple spermatophores may select against rapid female remating. Wingloading costs may be especially great at overwintering sites due to the long remigration distances required to reach oviposition sites. Either high costs or low benefits of female remating is inconsistent with the hypothesis that opportunity to maximize multiple mating accounts for dense overwintering aggregations.

Wells et al. (1990) noted that mating frequency increased at the California site just when the overwintering colony was becoming dramatically smaller and less dense due to the mass exit from the site. They suggested that the loss in benefit of high population densities is countered during dispersal from the overwintering site by the sharp increase in the sex ratio bias toward males that occurs during this time. I argue that the highly male-biased sex ratio during the highest frequency of mating is the primary determinant of the high number of female matings in California. The highly female-biased sex ratio observed in a late overwintering Mexico population (Chapter 2) is also consistent with the lower frequency and degree of polyandry there. Aggregated distributions are

expected to increase mating frequencies and may be a selective pressure for overwintering aggregations, but the data argue that this effect does not come through selection to maximize female nutrient or cardenolide benefits.

### Conclusions

The quantity of lipids transferred per mating, together with the degree of polyandry observed in Mexico overwintering monarchs, cannot account for the level of lipid content increase in late overwintering females in California observed by Wells et al. (1993). However, even the highest number of spermatophores recorded in monarchs (Suzuki and Zalucki (1986) could not account for the levels of lipid increases observed by Well et al. (1993). Similarly, cardenolide quantities obtained by multiple mating also appear to be an insignificant source of defensive chemical for females.

Although female lipid and cardenolide gains by multiple mating are apparently insignificant, other material benefits may be important factors leading to the high levels of polyandry in monarchs in other overwintering populations or during summer breeding conditions. However, regardless of the factors that have led to polyandry in monarchs, the extremely low frequency and degree of polyandry in the Mexico overwintering population suggests that opportunity to maximize multiple mating has not been a significant selective pressure in the evolution of extremely dense overwintering aggregations.

I suggest an opposite view from that of Wells et al. (1990) and Wells et al. (1993) with regard to the association of overwintering population structure and mating ecology: The spatial and temporal characteristics of the overwintering colony have been a significant factor in shaping monarch mating strategies. At overwintering sites environmental conditions, including the extreme population densities and a relatively brief and synchronized mating period provide an heightened opportunity for mating before dispersal. However, these same conditions may limit polygamy through environmental constraints in mating frequencies. Conflicts of interest between the sexes (see above, Knowlton 1979) may also limit mating frequencies under dense population conditions.

Other important ecological factors in shaping insect mating ecology include population age and spatial distribution, resource distribution, and sex ratio. Mexico, California, and Australia overwintering monarchs, and summer breeders show variation in mating patterns associated with variation in these parameters. Such variation during the different phases of the life history cycle and among populations is perhaps unsurpassed in any well-studied species. The monarch is therefore a unique single species comparative model for understanding how environmental constraints and opportunities shape insect mating ecology and the evolution and maintenance of a migration-overwintering life-history strategy.

Table 6-1. Wet weight, dry weight, lipid weight, percent lipid (of total dry weight), and cardenolide concentration of 30 mated pairs and fresh spermatophores just-transferred from the male to the female. Spermatophore cardenolide concentration was determined from 6 pooled samples of 5 spermatophores each (variation represents inter-batch variation).

Sample	Male Butterflies			Female Butterflies			Fresh Spermatophores		
	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range
Wet Weight (mg)	429	11	(277-527)	456	12	(354-614)	16.4	1.0	(8.1-28.9)
Dry Weight (mg)	181	6.6	(111-270)	195	6.6	(132-273)	5.2	0.3	(2.3-8.9)
Lipid Weight (mg)	32	3.7	(8.4-102)	42	4.2	(11.0-83)	0.7	0.1	(0.3-1.5)
Percent Lipid	16.4	1.3	(6.1-37.9)	20.6	1.5	(8.6-33.8)	14.9	1.4	(4.6-31.0)
Cardenolide Conc. (ug/0.1 g)	38.4	7.3	(0-138)	49.6	10.4	(0-266)	21.5	5.9	(0-38)

## CHAPTER 7

### DISCUSSION

Due to large male investments in spermatophores and their unusual life history strategy, monarch butterflies are especially useful study organisms for furthering our understanding of insect mating system ecology. This strategy includes an explosive mating event that follows reproductive diapause and occurs just before the long-distance remigration from extremely aggregated overwintering colonies. Unlike overlapping generations of summer breeders that mate in association with host plant distributions, mating at Mexico overwintering sites is far removed from larval resources in both space and time. The ecological conditions under which the explosive mating event occurs differs greatly from those of summer breeders.

I predicted that both male and female overwintering monarchs should show mate choice based on theoretical considerations and the following empirical evidence. First, males make large material investments in spermatophores (Oberhauser 1988, Svård and Wiklund 1988a). Second, females must remigrate long distances after depleting stored nutrients during the overwintering period (Masters et al. 1988, Brower 1985, Wells et al. 1993). Nectar sources are limited for female refueling before remigration. Third, females incorporate spermatophore-derived nutrients into their somatic tissues and egg production (Boggs 1981, Oberhauser 1992, Wells et al. 1993). Fourth, mating occurs during a temporally restricted

period, just before dispersal, and under extremely dense population conditions. These conditions should limit the reproductive rates of both sexes while heightening opportunity to exercise choice.

Both male and female monarch phenotype is expected to indicate their ability to provide the other sex with material benefits. Size is positively correlated, and age and mating history is negatively correlated with male ability to provide large nutritious spermatophores (Oberhauser 1988, 1989). Likewise, size is positively correlated, and age negatively correlated with female fecundity and possibility remigration ability (Campbell 1962, Suzuki 1978, Courtney 1982, Zalucki 1981, Rutowski 1985, Oberhauser 1995). Furthermore, multiply mated females may reduce male potential paternity assurance, even with last male sperm precedence. Large size, young age and virgin mating status are therefore predicted to be important cues for both male and female adaptive mate choice (Rutowski 1982, Rutowski 1985).

If both sexes prefer to mate with large individuals that are in good condition, and the ability to exercise choice is related to size and condition, (i.e., large, good condition males and females are better able to choose mates or resist unwanted mating attempts), positive assortative mating based on size and condition is expected. This prediction assumes that at least some females can avoid the advances of males during aerial pursuit and after takedown as males attempt to gain genital coupling (Crespi 1989).

In Chapter 2 I tested my prediction that overwintering monarchs should mate assortatively due both to male and female choice. *In copula* females showed phenotypic trends predicted by

sexual selection theory, i.e. those chosen by males were larger, heavier and in better condition than the females in the aggregated population. However, mating males were not of the phenotype expected to be selected either by male-male competition or by female choice: Small and worn males were over represented in mated pairs compared to those in the aggregated population.

In Chapter 3 I presented the results of my study that tested 2 hypotheses to explain the unexpected finding that small males had a mating advantage. I first experimentally tested whether small males might have an aerodynamic advantage in aerial pursuit of females. This has been found to occur in some other insects that mate on the wing. Instead, I found that large males had a higher likelihood of taking down females in aerial pursuit than did the small males. This advantage was eliminated in tests conducted in small cages in which the aerial component of precopulatory interactions was physically prohibited. In accordance with my field study (Chapter 2), I found no evidence of female choice when females had access to both large and small males.

I next tested whether the small male bias in Mexico resulted because large males in good condition do not attempt to mate under field conditions. Because butterflies of all sizes and conditions were present in flying, drinking and nectaring butterflies in and adjacent to the overwintering clusters, size-dependent differences in the motivation or ability to leave clusters or to take flight is unlikely to account for the small male mating advantage.

Since differential behavior did not account for the small size and poor condition bias, I next tested whether differences in the time

of post-diapause reproductive tract development could account for the small male mating advantage. If small, worn males break diapause sooner than large, good condition males, their reproductive glands should be heavier due to post-diapause development (Herman 1985). Contrary to my expectations, males in the general population were more likely to have well-developed reproductive tracts, while mating males were primarily old, with fragile tissues, and low reproductive tract tissue weights. They also had depleted abdomens, low lipid reserves and were in poor condition as evidenced by wing wear. Male reproductive gland weights in the general population, and in some mating males, was associated with increases in mating activity. Post-diapause reproductive condition may therefore account for some male overwintering behavior.

My results suggest that rather than female choice, physiological differences including 1) age, 2) size, and 3) post-diapause reproductive tract development interact to define the male mating population. Variation in male mating behaviors appear to represent condition-dependent mating tactics in which the costs and benefits of mating in Mexico versus after the remigration northward varies both with male age and size (Dunbar 1982).

Female monarchs incorporate spermatophore-derived nutrients into their somatic tissues and their eggs, they mate multiply, and they show strong, but incomplete last male sperm precedence (Oberhauser pers. comm.). Knowledge of when in the migratory life history cycle females receive spermatophores is therefore important in understanding 1) both male and female mating strategies, 2) remigration population demographics, and 3) the selective pressures



that shape and maintain the migration-diapause life history strategy. In Chapter 4, I therefore elaborated new techniques of bursa copulatrix dissection and abdominal palpation to determine when overwintering females had mated and the degree of polyandry that occurs during the different parts of the migratory cycle.

In Chapter 5, I framed the observed overwintering mating patterns within the ecological context of monarch overwintering natural history. My primary findings were that 1) contrary to generally held views, a substantial percentage of females mate during the southward migration and/or early in the overwintering period, 2) the mass mating event is restricted to the last few weeks of overwintering, increases exponentially, and most mating occurs just before or just after the main exodus from the overwintering site, 3) female utilization of spermatophores may increase as the mass mating event begins, 4) many females leave the overwintering site without mating during the mass mating event, but not necessarily as virgins, and 5) multiple mating during the mass mating is rare, but females that had mated on the way to the overwintering sites may mate again during the mass mating period.

Overwintering mating ecology observed at a single Mexican site over 4 years showed general similarities and some differences from the results of workers studying monarch mating behavior at overwintering sites in California and Australia. These are summarized in Chapter 5. In agreement with Herman (1993), differences among these populations appear to be a matter of degree.

California mating ecology appears to be generally intermediate between Australia and Mexico sites. Late in the overwintering

season, California and Mexico overwintering colonies apparently show opposite trends in sex ratio. Colonies are male-biased in California and female-biased in Mexico. This difference needs to be better substantiated through time and among years. Sex ratio is expected both to reflect and to define mating system ecology (Emlen and Oring 1977, Clutton-Brock and Parker 1992). Unraveling the causes of sex ratio changes through time is probably the key to understanding differences in the degree and frequency of female mating between Mexico and California.

I propose that variation in environmental conditions experienced among individuals, locations, phase of the life cycle, and among populations impose variation on monarch reproductive physiology and migration behavior. Variation arises through widespread geographical distribution and the protraction of adult eclosion in late summer overlapping generations. Differing environmental conditions are therefore experienced among individuals before leaving summer sites, during the southward migration, and during the overwintering period. Individual and sex differences in the costs and benefits of the time and frequency of mating are further defined by the distance from, and distribution of, larval resources (Emlen and Oring 1977, Odendaal et al. 1985). The resulting players and playing field, in turn, determine the frequency, degree, and evolutionary and demographic significance of overwintering female mating.

In Chapter 6, I re-evaluated the importance of male spermatophore investment as an important nutrient resource for females. Specifically, I addressed the hypothesis put forth by Wells

et al. (1990) and Wells et al. (1993), that female nutrient need has been a major selective force accounting for the extreme density of the overwintering colonies. Female nutrient needs may be an important determinant of female mating patterns. However, I found a low frequency of mating in the overwintering females, a low frequency of polyandry, and very limited quantities of lipids and defensive compounds in spermatophores. Increased opportunity for polyandry is therefore unlikely to be an important selective agent accounting for the extreme densities of Mexico overwintering aggregations. From the opposite perspective, I suggest that the extreme densities are an important factor shaping the monarch overwintering mating system. In fact, the extreme overwintering population densities may have been an important selective agent in the evolution of the male takedown mating tactic in which pheromones apparently play a reduced role in mate acquiescence.

Finally, I present a basic model illustrating these points in Figure 7-1. My model is consistent with what is known about monarch life history and mating biology. The sources of environmentally induced variation occur at various stages in the life history cycle (illustrated by ellipses) and lead to individual and population phenotypic variation in migration and reproduction. I assume that individuals may invest simultaneously in reproduction and migration, but that once reproductive they cannot reinstate diapause. The left and right hand cycles illustrate the resulting demographic impacts.

Starting with late summer generation, monarch phenotypic variation in size, age, and reproductive state arises through

developmental and neuroendocrine responses to temperature, daylength, and availability and quality of host plants, nectar sources and potential mates. Variation occurs both through time within a site and over the large geographic area covered by late summer generation monarchs. As noted by the relative size of the arrows, most butterflies enter the diapause/migration/remigration/mating cycle depicted by the right-hand cycle. Relatively fewer, but an environmentally variable proportion, enter the left hand cycle of reproduction/migration/transient aggregation and overwintering mating/remigration. As noted by the relative size of the arrows many of these are lost from the cycle due to their relatively large resource investments in reproduction. The reproductive success of those that move through the left cycle is divided into late summer broods and early spring broods, while diapausing male reproductive success occurs exclusively in the post-migration spring generation. Reproductive success of neither male phenotype is appropriately measured only at overwintering or early spring breeding sites.

During the southward migration and at temporary aggregation sites, some individuals will experience conditions conducive to mating. Depending on past experiences and on the intensity and length of summer-like conditions (as depicted by the left versus right hand transient aggregation phase), some monarchs will invest in mating. They may or may not gain reproductively and they may or may not continue migrating depending on their condition and the existing environmental circumstances (e.g., temperatures, sex ratio).

All diapausing and some reproductive monarchs will continue to overwintering sites. Again, depending on past experiences and

current conditions, some will mate at the overwintering site. As depicted by the left hand cycle, most summer breeders and some that experience summer-like conditions during the autumn migration will mate at the overwintering site. Relatively few of these will return during the remigration. Of those that overwinter in diapause (right hand cycle), more (e.g. California) or fewer (e.g. Mexico) will reproduce before migration, and/or again during remigration or at oviposition sites.

The relative proportions of the population moving through the right versus the left hand cycle as shown in Figure 7-1 is meant to represent eastern U.S. monarch population. Australian monarchs may be reversed with most entering the left hand cycle, and the western U.S. population may be intermediate between these. This model should help to make predictions of mating tactics in which the reproductive costs and benefits vary phenotypically among individuals, populations, and phases of the life cycle.

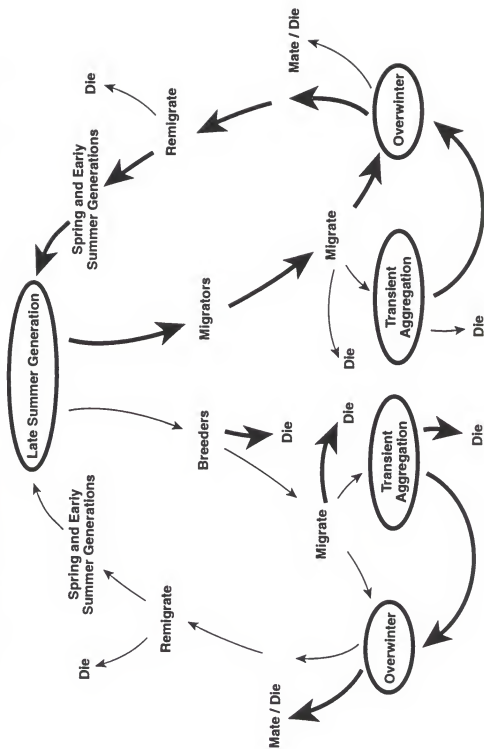
For example, understanding the adaptive significance of large male investments in spermatophores and female mating patterns must consider the monarch as a compromise of opportunities and constraints suited by environmental integration of physiology and behavior. Male monarchs takedown females that resist their mating attempts. Both male ability to overcome and female ability to resist may be related to their size, age, condition, and reproductive state. Females multiply mate, store sperm, and utilize spermatophore-derived resources. The last male to mate with a female gains most of the fertilizations, but sperm precedence is incomplete and may depend on the timing of the intermating interval. Mating is

separated from oviposition sites in overwintering, but not summer breeding, populations. Because the length and intensity of diapause differs between the sexes and is influenced by environmental conditions (Herman 1985), there may be differences among populations and among life cycle stages in which sex controls mating frequency. The relative degree of male-female conflict of interest is also expected to vary between winter and summer mating monarchs due to environmentally induced differences in nutrient budgets, spermatophore degradation rates, and intermating intervals (Boggs 1981, Drummond 1984, Oberhauser 1989). Furthermore, the appropriate measure of reproductive success cannot be measured by mating success due to female polyandry, and must include lifetime measures since males have reproductive options before leaving summer breeding grounds, during the autumn migration, at the overwintering site, during the remigration, and at spring oviposition sites.

In conclusion, my research demonstrates non-random mating at a Mexico overwinter site and emphasizes the importance of environmental conditions in determining both phenotypic-dependent mating behaviors and their reproductive consequences. I encourage comparative studies of monarch mating patterns during different stages of the life history cycle and in different populations to determine the evolutionary and demographic effects of these mating patterns.

Figure 7-1. A basic model of sources of variation in monarch migration/overwintering mating strategies and the implications for repopulation potential and demographics. Heavy lines indicate that relatively larger proportions of monarchs generally move in the indicated direction as compared to those moving in the direction indicated by the thin lines. Encircled areas are monarch populations found during different phases of the migratory life cycle. Environmental conditions experienced before and during these phases are sources of variation in the relative proportions of individuals moving to the next phase. Variation arises as neuroendocrine responses to temperature, daylength, and availability and condition of host plants and nectar sources. Genetic flexibility (e.g. diapause versus oligopause) and environmental conditions encountered interact to determine these neuroendocrine responses in individuals. In this model, once a monarch becomes reproductive, it can no longer reinstate diapause, but it can migrate. Also, mating is at least potentially divorced from the presence of developed gonads (i.e. southward migrating monarchs that mate may or may not reproduce during that phase). Genetic and environmental differences among eastern and western U.S. monarch populations and Australian populations may lead to differences in mating tactics among these populations and also among different parts of the breeding-migration-overwintering-remigration cycle. These, in turn influence population demographics and genetic structure of the spring first generation summer breeders. See text for details.

### Fates of Monarchs in their Annual Migration / Reproduction Cycle





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## BIOGRAPHICAL SKETCH

Tonya Van Hook was born on 18 June 1959 in Terre Haute, Indiana. A youth spent in the rural woodlands of Indiana nurtured a respect and wonderment of nature and has been a guiding force in her academic and personal endeavors. Florida has been her home since she moved there as a young teenager. She currently lives in Tallahassee with her husband, David Kirschke, MD., and their dog, Sweetpea.


Tonya received an interdisciplinary Bachelor of Science degree at the University of Florida in 1986, combining entomology, zoology and psychology in the study of insect behavior. In the fall of 1986, Tonya began graduate studies in the Department of Entomology at the University of Florida under the guidance of Dr. Lincoln Brower and Dr. John Sivinski. Her graduate work focused on insect ecology and behavior.

The time spent doing her 4 years of field work in the remote mountains of Sierra Chincua, Mexico, crystallized her current interests in conservation biology. Tonya hopes to use insects to promote the valuation, enjoyment, exploration, and preservation of nature. Her career goals are to promote the use of insects as conservation tools, to contribute to better integration of agriculture and conservation goals, and to demonstrate the value of insects in environmental education.

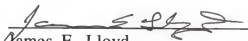
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John M. Sivinski, Chair  
Assistant Professor of Entomology  
and Nematology

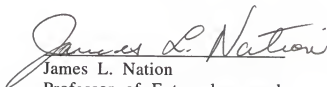
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Lincoln P. Brower, Cochair  
Distinguished Service Professor  
of Zoology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

  
James E. Lloyd  
Professor of Entomology and  
Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

  
James L. Nation  
Professor of Entomology and  
Nematology

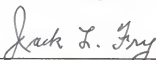
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Jonathan Reiskind  
Associate Professor of Zoology

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December 1996

  
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